BIOPHYSICAL AND BIOCHEMICAL STUDIES ON THE TRANSPORT ACROSS PERICARDIUM

DISSERTATION
Submitted to the Aligarh Muslim University, Aligarh
for the degree of

Master of Philosophy

IN

CHEMISTRY

BY

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1986

Dedicated to

my parents, a motivating source of light behind whatever little success I have achieved.

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Dated: 22.10.1986

C E R T I F I C A T E

This is to certify that the dissertation for M.Phil. entitled, "BIOPHYSICAL AND BIOCHEMICAL STUDIES ON THE TRANSPORT ACROSS PERICARDIUM" embodied the work carried out by MR. HAROON RASHID KHAN under our supervision.

He has fulfilled all the requirements for the Degree of MASTER OF PHILOSOPHY (M.Phil.) in Chemistry, regarding the nature and period of the investigational work.

The work included in this dissertation has not been submitted for any other degree and unless otherwise stated, is all original.

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ACKNOWLEDGEMENT

Firstly let me thank to Allah, the Almighty, for it is due to Him only that this work has been completed amidst a number of hurdles and obstructions.

I am greatly indebted to my supervisor, Dr.Hasan Arif, Reader Department of Chemistry, A.M.U.Aligarh for his generous help and skilful guidance during the course of preparation of this dissertation.

I will be failing in my duty if I don't mention here the name of my co-supervisor Prof.(Dr.) Mohammad Ahmad, Superintendent,J.N.Medical College Hospital,A.M.U.Aligarh,for whatever merit this dissertation deserves is due to his time to time advice, constructive criticism and untiring help.

My sincere thanks are also due to Prof.Mehdi Hasan, Principal, J.N.Medical College, A.M.U.Aligarh, for his valuable suggestions and sincere guidance.

I am thankful to Prof. M.S.Ahmad, Chairman, Department of Chemistry,A.M.U.Aligarh for providing necessary facilities.

I am also thankful to I.C.M.R.,New Delhi for providing financial assistance.

Thanks are due to Mr. Irshad, the chemical supplier, without whose generous effort, this work would have completed much earlier.

I wish to take this opportunity to thank all my friends and well-wishers for their inspiring assistance and valuable suggestions throughout the completion of this dissertation. To name a few who always multiplied my joys and pleasures and divided my pains and sorrows; P.Sultan, J.Sultan, Kamaluddin, M.A.Salam, Shahzad, Noor Alam, Tariq Saeed, Tariq Khan, P.Khalid, Shoaib, Shamshad, Fazal, Zaman, Sanjiv, and Imteyaz.

I am here bound to thank to Mr.Shahid Jamal Khan, who always tendered his help whenever I need it the most.

Unqualified praise are due to my uncle Mr.Rahmatullah Khan for his constant persuation to continue my higher studies.

My special thanks to my brothers, Mr.Inamullah Khan, Mr.Zafrullah Khan and my uncle Mr.Yusuf Khan, Whose unending criticism gave a boost to my morale.

With love and affection, I like here to mention the sharing of pleasant, exciting and exhilarating moment of my life by my sisters and younger brother, Rizwan.

The whole story will remain incomplete if I don't express the indebtness to my parents, who, as I believe will be the happiest sole on this earth to see my work completed. I know their affection, love, guidance, patience can never be paid back by mere thanks.

(HAROON RASHID KHAN)

C O N T E N T S

REVIEW OF LITERATURE

The study of transport phenomena across biological membranes during the last few decades has acquired much importance. This field has attracted the attention of chemists, physicists, chemical engineers, pharmacologists, biochemists, physiologists and biologists because of great applied significance of biological membranes. To understand the behaviour and properties of complex cell membranes, an extensive work is available. The work related to these field are contributing significantly in terms of established physio-chemical principles. The literature pertaining to membrane phenomena has been reviewed in a number of books and publications (1-8). Many investigators have worked related to the studies on trace metal elements (9,10) in body tissues, purification of biological membranes (11), transport processes in membranes (1,5,12,13), Histopathological properties including electron microscopic studies (14-16) and biophysical properties of model membrane (17-20).

SIGNIFICANCE OF THE PRESENT STUDY

Pericardium has received great significance during the last several years because of their useful role in production of cardiac valves (21-24). These bioprosthetic valves are expected to prove superior to artificial valves because these will not required long term anticoagulant therapy. Such valves

are of special significance in our country because of lesser financial implication in their manufacture and maintenance.

It has been proved from clinical follow-up of patients that certain bioprosthetic cardiac valves (procine valves) get calcified with the passage of time. Altered calcium metabolism has been implicated in the production of calcification. Attempts have also been made to examine the electron microscopic and Histopathologic alteration in calcified procine cardiac valves (23, 24).

Metals are important constituents of all biological tissues, including the biological membranes. They effect the biophysical properties of biological membranes by virtue of electrophysiological effects. They are also important components of several metalloenzymes. Therefore, they also effect the active transport through the membranes by influencing the various membrane enzymes. Pericardium has two important functions (i) Provision of physical protection to heart, and (ii) control of active movement of fluid through these membranes. Therefore, it is important that alteration in metal environment will not only influence the electrophysiological properties of these biological membranes but will also influence active transport through them by altering the function of the various membrane enzymes of which the metals are important constituents. These studies are important not only in understanding of the various biological functions of these biological membranes, but

will also help in understanding the mechanisms which will influence the long term prognosis of the cardiac valves prepared from these biological membranes.

PERICARDIUM

The pericardium (Fig. A) contains the heart and the roots of the great vessels. It is placed in the mediastinum behind the body of the sternum and the cartilages of the ribs from the second to the sixth inclusive, and infront of the thoracic vertebrae, from the fifth to eighth inclusive. It is essentially composed of two opposed layers of serous membrane, separated by a mere film of fluid, which nevertheless provides a complete cleavage between the heart and its surroundings allowing it, freedom of movement within the pericardium.

The pericardium consists of an outer sac, known as the fibrous pericardium, consisting of fibrous tissue and a inner, double layer sac, the serous pericardium, a delicate membrane which lines the fibrous sac and covers the heart.

The fibrous pericardium is a cone-shaped bag, the apex of which is considered to end where it is continuous with the external coats of the great vessels, while its base is attached to the central tendon and to a small part of the muscular substance of the left half of the diaphragm. The fibrous

Fig. A: The interior of the pericardium viewed from in front.

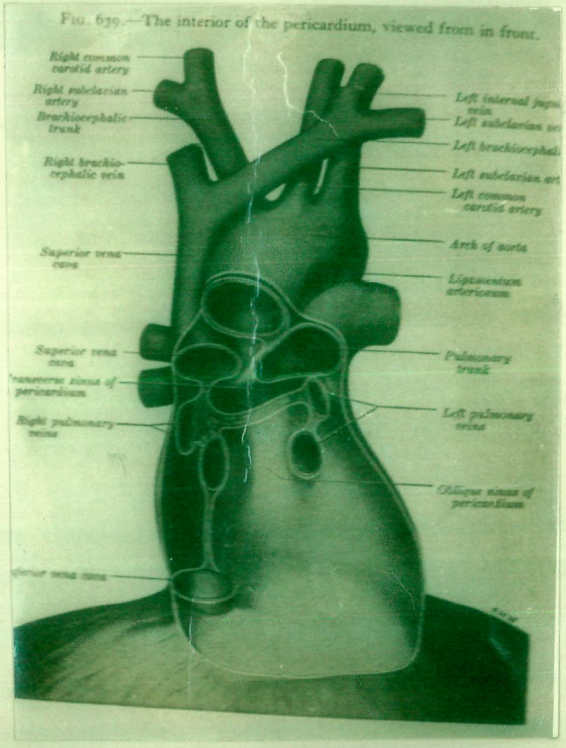


FIG. 639.—The interior of the pericardium, viewed from in front.

percardium is also attached to the posterior surface of the sternum by superior and inferior sternopericardial ligaments, the superior passing to the upper end of its body, and the inferior to its lower end.

Anteriorly, the fibrous pericardium is separated from the front wall of the thorax, in the greater part of its extent, by the left half of the lower part of the body of the sternum and the sternal ends of the cartilages of the fourth and fifth ribs of the left side is in direct relationship with the chest wall. Posteriorly the fibrous pericardium rests upon the principal bronchi, the oesophagus, the oesophageal plexus of nerves, the descending thoracic aorta and the posterior part of the mediastinal surface of each lung. Laterally, it is separated by the pleura, from the mediastinal surfaces of the lungs; the phrenic nerve, with its accompaying vessels, descends between the fibrous pericardium and the mediastinal pleura on each side, inferiorly, it is separated from the liver and the funds of the stomach by the diaphragm.

The serous pericardium is a closed sac which lines the fibrous pericardium and is invaginated by the heart, it therefore, consists of a visceral and a perietal layer. The visceral layer, or epicardium, covers the heart and the great vessels, and from the latter it is reflected to form the perietal layer, which lines the fibrous pericardium. (25)

STRUCTURE OF PERICARDIUM

The fibrous pericardium consists of a compacted network of a collagenous fibrous tissue. The serous pericardium consists of a single layer of flattened cells resting on a layer of subserous aerolar tissue which, in the case of perietal layer, blends with the fibrous pericardium. The areolar tissue under the visceral layer is continuous with the intestinal tissue of the myocardium and contains fat which is greatest in amount along the ventricular border of the coronary sulcus, along the inferior border of the heart and in the interventricular grooves (25).

STRUCTURE OF BIOLOGICAL MEMBRANE

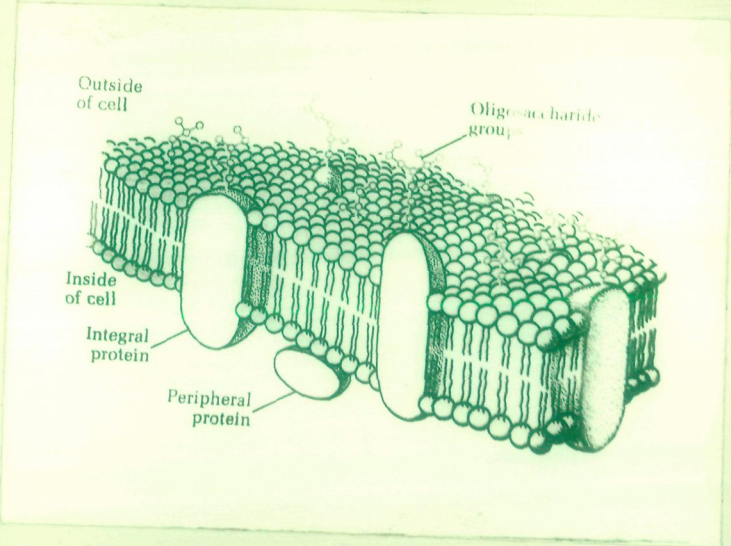
Membranes in general may be broadly classified as natural and artificial membranes. Natural membranes existing in biological systems (biomembranes) are considered to have a fundamental unit membrane structure which is a biomolecular leaflet of lipid with their polar groups oriented towards the two aqueous, the extracellular and the intracellular, phases of the cell; protein is supposed to exist close to the polar heads of the leaflet (26-28).

Since the natural membranes are found in living cells, and therefore, have applied importance. It is much interesting to understand the structure function and behaviour of natural

polar lipids, with their hydrocarbon chains oriented in-wards to form a continuous hydrocarbon phase and their hydrophilic heads oriented outward. Each surface was thought to be coated with a monomolecular layer of protein molecules, with the polypeptide chains in extended form. Later other investigators proposed globular or subunit model, in which membranes were viewed as consisting of sheets of recurring lipoprotein subunits of diameter 4.0 to 9.0 nm, resembling the subunit structure of some oligometric proteins or the coats of some viruses. However, globular models have failed to account satisfactorily for many properties of membranes.

S.J. Singer and G.L. Nicolson(34), postulated the most acceptable and satisfactory model of membrane structure to date appears to be the fluid mosaic model. This model (fig. B) postulates that the phospholipids of membranes are arranged in a bilayer to form a fluid, liquid-crystalline matrix or core. In this bilayer individual lipid molecules can move laterally, endowing the bilayer with fluidity, flexibility, and a characteristically high electrical resistance and relative impermeability to highly polar molecules. The fluid-mosaic model postulates that the membrane protein are globular, to account for their high content of α hel-ix. The various membrane proteins would form a mosaic-like structure in the otherwise fluid phospholipid bilayer. This mosaic is not fixed or static.

Fig. B: The Singer - Nicolson fluid mosaic model of membrane structure.


Outside
of cell
Oligosaccharide
group
Inside
of cell
Integral
protein
Peripheral
protein

The fluid mosaic model accounts satisfactorily for many features and properties of biological membranes. It provides for membranes with widely different protein content, depending on the number of different protein molecules per unit area of membrane; it provides for the varying thickness of different types of membranes; it can account for the electrical properties and permeability of membranes and it also account for the observation that some protein components of cell membranes move in the plane of the membrane at a rather high rate (2).

Rouser and co-workers(35) have summerised their considerable analyses of many membrane systems as follows:

1) All animal cell membranes contain phospholipids. The same classes of phospholipid are found in vertebrates and invertebrates. Some membranes (e.g. myelin) contain glycolipids whereas others donot. Only certain membranes contain sterol.

2) Plasma membranes, (cell surface) of the endoplasmic reticulum, nuclear membranes and mitochondrial membranes from the same species have different compositions. All differ quantitatively and to some extent qualitatively in the classes of lipid present. For example, plasma membranes or elaboration of these appear to contain most of the glycolipid of the cell.

3) The proportion of the different phospholipids vary greatly and the total amount as well as the types of both

ceramide polyhexosides and gangliosides is very different in different species. Data from whole organs indicate that plasma membranes from different cell types of the same species may vary in composition.

4) The fatty acid composition of each class of lipids from different organelles and organs of one species, as well as from different species, is variable. This is true even when the classes of lipids are the same in the different structures. Individuality is thus expressed most clearly in differences in fatty acid composition.

COMPOSITIONS

Most of the membranes contain about 40 per cent lipid and 60 per cent protein, but there is considerable variation. At one extreme the inner mitochondrial membrane contains only about 20 to 25 percent lipid, and at the other the myelin membrane surrounding certain nerves may contain upto 75 percent lipid. The lipids of membranes are largely polar, phosphoglycerides, predominate, with much smaller amounts of sphingolipids. In fact, nearly all the polar lipids of cells are localized in their membranes. Endoplasmic reticulum and organelle membranes contain relatively little cholesterol or triacylglycerol, whereas the plasma membrane of some cells of higher animals contains much cholesterol, both free and esterified.

Membrane can be classified in two categories. The extrinsic or peripheral, proteins are only loosely attached to the membrane surface and can easily be removed in soluble form by mild extraction procedure. The intrinsic or integral, proteins, which make up 70 percent or more of the total membrane protein are very tightly bound to the lipid portion and can be removed only by drastic treatment (34).

The isolation and characterization have been studied by the histocompatibility antigens of plasma membranes (36-39). These molecules are glycoproteins and are exposed on the outer surface of the membranes. Possibly, they are associated with membrane particles (40) as are the glycoproteins of the erythrocyte membrane. Two main glycoproteins are found by SDS-gel electrophoresis in the membranes of rat liver and kidney cells (41), Hela cells (42), hamster kidney fibroblasts (43), and mouse liver cells (44) of membranes. Electron microscopy has revealed that membranes have a trilaminar structure with a total thickness between 7.0 and 9.0 nm, depending on the type of membrane.

The cell is a locus of chemical structure and function in which a continuity of proporties is maintained in the midst of drastically different and ever changing environment. One important mechanism by which the cell achieve this constancy, is the regulation of movement of material into the cell and out of

it even within the cell, materials are not uniformally distributed. Here also a precise regulation of interchange of materials is encountered. To achieve this regulatory control the cell utilizes its delicate membrane of about 75 ^{o}A thick.

TRANSPORT PHENOMENA THROUGH BIOLOGICAL MEMBRANES

At one time it was thought that the movement of substances through membranes was determined solely by concentrations gradient, but movement against the concentration gradient has been observed in most biological tissues or biomembranes, e.g. K is usually accumulated in plant and animal cells to a concentration many times higher than that of the medium surrounding the cell. Such transport requires energy by the cell and has been called the active transport, while that which responses to a concentration gradient is a passive transport. Kinetic energy accounts for random movement of the molecule and the chemical potential energy of higher concentration of a particular substance outside the cell than inside the cell. This gives effective direction to the movements, is dissipated as the molecule of that substances outside of the cell move into the cell and reduces the concentration gradient of substances between the outside in different ways by various authors but no satisfactory definition has been given as yet (45).

The specific transport of ions is a common function of a biomembrane. An active transport process is usually defined as one that can bring about a flow of a substance against an electrochemical potential gradient of the substance, the name implies that specific biological activity is involved in the process described by Rosenberg(46). It means that the dissipation function is smaller than zero. This is observed in the active and selective transport of metal ions through a protoplasmic liquid (47-49).

The general features of carrier transport systems, both equilibrating and transporting uphill, were reviewed by Wilbrandt and Rosenberg (50) and compared to reported observations. It was pointed out that a number of kinetic features are shared by "absorption systems" which involve binding of substrate at fixed sites rather than on mobile carriers, but that counter transport and competitive activation are criteria for mobile binding sites. A number of pertinent observations reported in the literature were discussed (51). Recent observations mentioned here concerning counter transport (52-55) which were not interpreted by some authors along the same lines, are also pertinent in this context.

Katchalsky et al. (56,57) elaborated their description of biological transport processes in terms of irreversible thermodynamics. For biologists with limited mathematical

sophistication Katchalsky (58) has summarized the most important aspects of his work. A number of consideration based on the concept of irreversible thermodynamics have been entertained.

Rosenberg and Wilbrandt (59) correlate facilitated diffusion with active transport by assuming a conversion of a "nonactive" carrier to an "active" one.

The transport system for mamalian tissues appear to be constitutive and firmly bound to the membrane. In general the transport systems for most of the aminoacids in microorganism are constitutive, while for sugars only glucose transport activity appears to be constitutive (60). The rest of the sugar transport systems are inducible.

Earlier kinetic studies on animal cells provided evidence for membrane mediation of solute transport (61). The nature of the membrane components, which have been termed "carriers", is not well defined. These kinetic studies have, however, provided us with a description of the number and kinds of transport systems present in the membrane as well as a basis for recognition of the receptor sites (62).

A number of studies on perfused hearts of rats were reported from Park's laboratory (63,64) and summarized by Morgan, Post and Park (65). It was found that insulin increases the rate of glucose uptake several fold.

The important relationships between the alkali metal ions and the physiology of animal cells have long been recognized. Most animal cells have an active $Na^+ - K^+$ transport systems which allow them to maintain high levels of intracellular potassium ions and low levels of sodium ions (66).

A very extensive coverage of the subject of the ($Na^+ - K^+$) ATPase and alkali metal ion transport has been made by Bonting (67). Additional reviews covering this area are the following Heinz (68), Albers (69), Post (70), Whittam and Wheeler (70a).

Transport of calcium into vesicle preparations of sarcoplasmic reticulum has been extensively studied by Hasselbach and Makinose (71, 71a), Kana-Zawa et al (72). These vesicles contain a magnesium and ATP-dependent transport systems for calcium which can produce and maintain large calcium gradients.

Zerahn (73) and co-workers have designed a method for direct study of Li and Na content of frog skin. "Ussing (74) at Harvey lecture summarized his work about the water and electrolyte transport in frog skin. He considered epithelial layer of frog skin as a two membrane system where Na^+ enters the first living cell layer by a sodium selective membrane. From this layer the Na either flow into next cell layer via cell "bridge" or can extrude into the interspaces which is open towards the inside. The basal membrane represents another tight resistance to free flow of Na and is the site of the pump.

Chock and Titus (75) gave an idea of mechanism of ion transport through biological and artificial membrane and also discussed the effect of alkali cation on enzymic activity and Na dependent transport of polar organic substances. Larsen and Rasmussen (76) studied about the role of membrane potential for chloride transport across toad skin. Hernandez et al. (77) measured the apparent transport numbers for a homogeneous passive membrane separated by binary electrolyte (NaCl). Torres et al. (78) carried out the electrophysiological characterization of ion transport (Na^+ and $C\bar{l}$) across isolated frog skin. Navebska, Koter and Kujawski (79) studied the isothermal transport of ions across the perfluorinated Nafion membrane in contact with NaCl, on the basis of irreversible thermodynamics transport. De—villarde et al. (80) measured the membrane potential and deduced the ionic transport numbers inside the proteic phases. By analyzing the assymetric membrane potential, Nakagaki et al.(81) got an information on the assymetry of the membrane structure. Santha Kumari, Sheela and Radha-Krishna Murty (82) reviewed the mechanism of ion transport across cell membrane with reference to nerve cell. Ussing (83) determined the emf. of active Na transport in skin epithelium with the help of steady state flux equation. Zeevi, Ameera et al. (84) discussed the selective transport of Li^+ across lipid bilayer membrane. Lauger et al. (85) measured the electrical properties e.g. capacitance,

Resistance and conductance of bimolecular phospholipid membranes. Benavente (86) measured membrane potential of porous membranes and calculated apparent transport number of cation from diffusion potential equation within the frame work of thermodynamic of irreversible process.

IMPORTANCE OF TRACEMETALS

It is becoming increasingly evident that trace metals as an integral part of tissues and biologic fluids are one of many homeostatic mechanisms regulating the reactivity of tissues and cells. Several of these actions are mediated through membrane bound enzyme system (87). However, the investigations in this direction have been confined to the study of few enzymes only (e.g. ATPase, NADH) mainly in liver tissue and blood cells. Furthermore, the effect of interaction of trace metals on these enzymes have not been investigated. Recent studies have revealed that changes in trace metal concentration were closely related to a number of disease in human and plant systems. The studies of the effect of trace metals on bio-membranes are therefore, important in a country like ours where marked variation in trace metal concentration is observed in soil, water and indigenous food stuffs.

The trace elements (Zn, Fe, Co, Ni, B, Al, V, Mo, Si, Sn, Cr, etc.) required in very small amount i.e. in milligram

or microgram only. Some 15 trace elements are known to be required in animal nutrition.

Most of the essential trace elements, function as enzyme co-factors or prosthetic groups. Essential elements appear to function in such enzymes in one of atleast three different ways: (a) The essential element may already have inherent activity in catalyzing a chemical reaction, which is greatly enhanced by the enzyme protein. This is specially true of the metals iron and copper. (b) The essential metal ion may form a complex with both the substrate and the enzyme active site, thus bringing them together in an active form. (c) An essential metal ion may function as a potent electron withdrawing agent at some point in the catalytic cycle.

Iron is among the best known of the trace elements with regard to biological function. Iron is a component of the heme groups of the oxygen-carrying proteins haemoglobin and myoglobin and of the electron carrying mitochondrial protein cytochrome. Iron is also present in catalase, which catalyses the decomposition of hydrogen peroxide, and in peroxidase, which catalyses oxidation of various organic substances by peroxides. Iron atom itself is an active participant in the catalytic cycle.

Copper plays an important role in the catalytic activity of cytochrome oxidase; copper is also present in the active group of lysyl oxidase. Animals that are copper-difficient

develop defective collagen molecules lacking cross-links, with the result that the collagen and elastin in the walls of major arteries become weakened and the arteries tend to rupture. Copper is also required for the proper utilization of iron in the body.

Zn^{2+} is an essential component of nearly a hundred different enzymes. It is present in many NAD - and NADP-linked dehydrogenases, enzymes that promote the transfer of hydride ions from substrate molecules to the coenzymes NAD^{+} and $NADP^{+}$. It participates in important enzymatic reactions involved in the replication and transcription of genetic information. The most interesting role of Zinc is the proper functioning of the taste and small receptors of the tongue and nasal passages.

The enzyme arginase, which hydrolyzes arginine to form urea, and end product of human amino group metabolism, contains tightly bound Mn^{2+}, which is essential for its activity. Mn^{2+}, also serves as a co-factor of some phosphate-transferring enzymes (88).

THEORIES

Various theories have been proposed from time to time to account for the permeability, phenomena in membrane.

Michaelis (89) and his co-workers in twenties and early thirties has tried to characterize the permeability of dense membrane in terms of electrical potentials measured in a solution-membrane solution system.

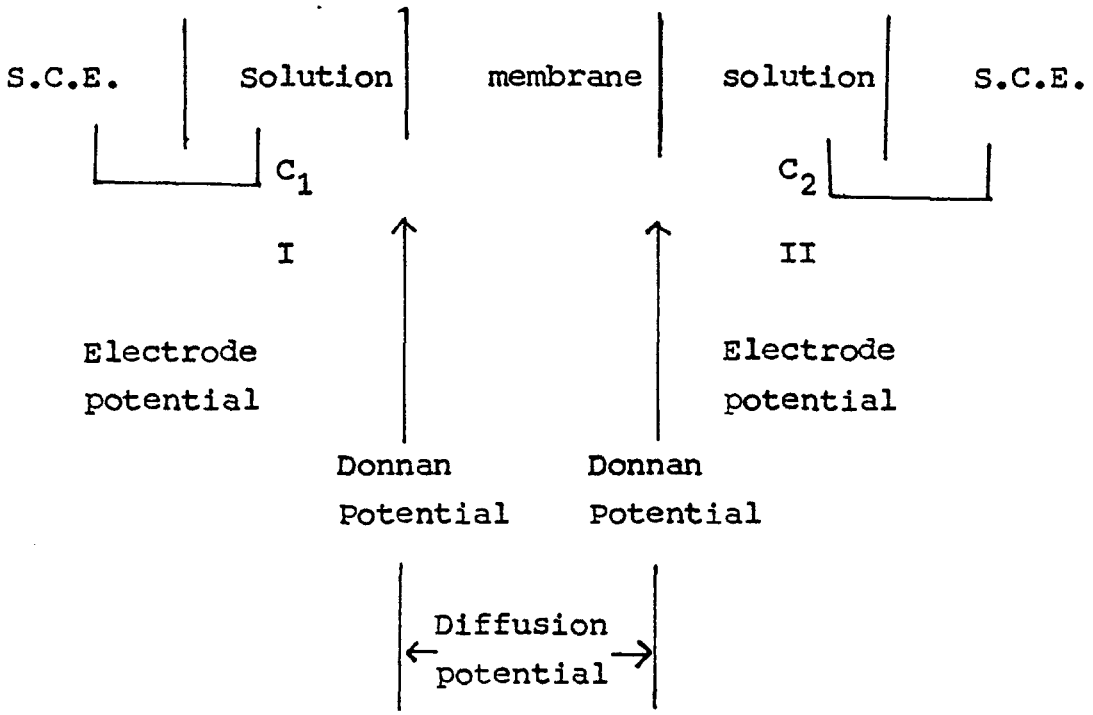
Teorell (90) and later Meyer and sievers (91) independently put forward identical theories which assume that the membrane itself has a fixed charge due to either absorption or dissociation. The physical essence of the fixed charge theory of Teorell, Meyer and siever's (TMS) can be stated qualitatively in a following way.

According to the fixed charge theory the walls of the pores of the membranes carry internally a definite number of potentially dissociable groups; anionic (acidic) groups such as carboxylic groups in case of electronegative membrane and cationic (basic) such as amino groups in the case of electropositive membranes. These dissociable groups are an integral part of the membrane structure.

According to Teorell (92) the essential feature of the original fixed charge theory was, the assumption that overall membrane potential was composed of three potential jumps; two Donnan potentials at each solution-membrane interface (here denoted by $\pi 1$ and $\pi 2$) and one residing inside the membrane (the internal potential or diving potential denoted by $\phi_2 - \phi_1$). The total membrane potential (Em) is given by:

$$Em = (\pi 1 + \pi 2) + (\phi_2 - \phi_1)$$

The electrical potential arising across an ionic membrane separating different salt solutions are usually measured by constructing the cell of the type:



The theoretical approaches made to calculate the emf's of cell described above according to Lakshminarayanaiah (93) fall under three groups.

1) The idealized theory of T.M.S. (90-92) and its refinements (113, 114).
2) The pseudo thermodynamic approach due to scatchard (94,95) and the treatment based on the thermodynamics of irreversible processes (96-99).
3) A kinetic approach based on the theory of absolute reaction rates (100, 101).

The earliest systematic measurements of membrane potential were made by Michaelis (102) and were later considerably added to by Sollner and Gregor (103), Marshall [104], and by Marshall and Ayers (105) who used colloidian and zeolite membranes, respectively. Wyllie and patnode (106) used heterogenous membranes made by imbedding ion exchange resin beads in inert binders and measured membrane potentials.

The work described in this dissertation is mainly concerned with the transport studies through the membrane and potentiometric evaluation of membrane charge density. The methods used are (a) Kobatake et al. (18, 99, 107, 108) and (b) Nagasawa et al. (20) based on the thermodynamics of irreversible process. Brief account of the various theories involved in (a) and (b) methods are given below.

Kobatake et al. (99) deduced the following equation for the electric current density I_c, relative to the frame of reference fixed to the membrane, using the basic flow equations provided by the thermodynamics of irreversible processes.

$$I_c = F\left(\bar{1}_+C_+ + \bar{1}_-C_-\right)\frac{d\phi}{dx} - RT\left(\bar{1}_+C_+\frac{d\ln a_+}{dx} - \bar{1}_-C_-\frac{d\ln a_-}{dx}\right) + F\left(C_+ - C_-\right)Um. \quad \text{----------(1)}$$

Here ϕ is the electric potential, C_+ and C_- are concentrations of +ve and -ve ions in moles percubic centimeter of solution, a_+ and a_- are activities of positive and negative ions

in moles per cubic centimeter of solution, 1_+ and 1_- are molar mobilities of +ve and -ve ions defined in terms of the mass fixed frame of reference, Um is the velocity of the local centre of mass, R is the molar gas constant, T is the absolute temperature of the system and F is the Faraday constant.

For the evaluation of Um, the viscous force acting on $1cm^3$ of solution in the membrane is represented by (1/K) Um, where K is a constant. The same volume of solution undergoes an electric force which is represented by

$$-F\ (C_+ - C_-)\ \left(\frac{d\phi}{dx}\right) \qquad \text{---------- (2)}$$

In the steady state, the sum of these two forces is zero, so that

$$Um = -KF\ (C_+ - C_-)\ \frac{d\phi}{dx} \qquad \text{---------- (3)}$$

Kobatake et al. (99) for convenience have considered a membrane which is ionized negatively with a charge density (in moles/ cc). Then the requirement that the electric neutrality must be realized in any element of the membrane gives the relation

$$C_+ - C_- = \theta \qquad \text{---------- (4)}$$

Since in the system considered here, no electric current is applied externally across the membrane, no net charge is transported from one side of the membrane to the other. This

means that I_c must be zero at a cross section of the membrane. Substituting eq. (3) and (4) into eqn. (1), putting I_c equal to zero, and solving for $\frac{d\phi}{dx}$ the following expression is obtained.

$$\frac{d\phi}{dx} = \frac{-(\frac{RT}{F})\left[l_+ (C_- + e) \frac{(d\ln a_+)}{dx} - l_- C_- \frac{(d\ln a_-)}{dx}\right]}{(l_+ - l_-) C_- + l_+ e + KFe^2} \quad \text{------ (5)}$$

To proceed further, the activities a_+ and a_- must be known as function of C_-.

Assumptions for a_+ and a_-

Kobatake et al. have assumed the following relation

$$a_+ = C_-$$

$$a_- = C_- \quad \text{---------- (6)}$$

and $$\gamma_+ = C_- / (C_- + e)$$

$$\gamma_- = 1 \quad \text{---------- (7)}$$

where γ_+ and γ_- are the activity coefficients of +ve and -ve ions in the membrane.

Equation For Membrane Potential

With eqn. (6) and (7) assumed for a_+ and a_-, eqn. (5) becomes

$$\frac{d\phi}{dx} = -(\frac{RT}{F}) \frac{(l_+ - l_-) C_- + l_+ e}{\left[(l_+ + l_-) C_- + l_+ e + KFe^2\right] C_-} \left(\frac{dc_-}{dx}\right) \text{----(8)}$$

when the bulk solution on both sides of the membrane is vigrously stirred, no potential gradient is set up, so that the desired membrane potential $\Delta\phi$ is obtained by integrating $\frac{d\phi}{dx}$ over the thickness of the membrane, We have

$$\Delta\phi = -(RT/F)\left(1/\beta \ln C_2/C_1 - (1 + 1/\beta - 2\alpha) \times \ln \frac{C_2 + \alpha\beta\theta}{C_1 + \alpha\beta\theta}\right) \qquad \text{-------(9)}$$

where $\alpha = 1_+/1_+ + 1_-$ -------(10)

$\beta = 1 + (KF\theta/1_+)$ -------(11)

and parameters have been assumed to be independent of salt concentration.

Kobatake et al. (99) have derived two useful limiting forms of eqn. (9). When C_2 becomes sufficiently small with Υ fixed; eqn. (9) may be expanded to give eqn. (12):

$$|\Delta\phi_r| = 1/\beta \ln\Upsilon - \frac{\Upsilon - 1}{\alpha\beta\Upsilon}\left(1 + 1/\beta - 2\alpha\right) C_2/\theta \qquad \text{-------(12)}$$

where $|\Delta\phi_r| = F\Delta\phi/RT$ -------(13)

It has also been shown by Kobatake et al. that at a fixed Υ , the inverse of an apparent transport number t_{app} coionspecies in a negatively charge membrane is proportional to the inverse of the concentration C_2 in the region of high salt concentration. Here t_{app} is defined by the relation

$$|\Delta\phi r| = (1-2\,t_{app})\,\ln\gamma \qquad \text{----------} \quad (14)$$

substituting for $\Delta\phi$ from eqn. (9) and expanding the resulting expression for $1/t_{app}$ in powers of $1/C_2$ gives eqn. (15)

$$1/t_{app} = 1/(1-\alpha) + \frac{(1+\beta-2\alpha\beta)\,(\gamma-1)\alpha}{2\,(1-\alpha)^2\,\ln\gamma}(\theta/C_2) + \text{-------}(15)$$

Kobatake et al. (99) employed another eqn. (16) for membrane potential, starting with the basic flow equation provided by the thermodynamics of irreversible processes and using a different set of assumptions, namely: (a) the contribution of mass movement is negligible (17) and (b) small ions donot behave ideally in charged membrane.

$$\Delta\phi = -\frac{RT}{F}\ln\frac{C_2}{C_1}(2\alpha-1)\ln\frac{\sqrt{4C_2^2+\phi^2x^2}+(2\alpha-1)\phi x}{\sqrt{4C_1^2+\phi^2x^2}+(2\alpha-1)\phi x}$$

$$-\ln\frac{\sqrt{4C_2^2+\phi^2x^2}+\phi x}{\sqrt{4C_1^2+\phi^2x^2}+\phi x} \qquad \text{--------}(16)$$

where ϕ is a characteristic factor of the membrane electrolyte pair and represents fraction of counterions not tightly bound to the membrane skeleton. The product ϕx is termed the thermodynamically effective fixed charge density of a membrane. Kobatake et al. (99) have proposed a simple method using the following approximate eqn. for the diffusive contribution to the

emf of a cell with transport.

$$\Delta\phi = -\frac{RT}{F}(1-2T_{app})\ln\frac{C_2}{C_1} \qquad (17)$$

where T_{app} is the transference number of ions in the membrane phase, Comparison of eqns. (16) and (17) gave,

$$T_{app} = \frac{1-2\alpha}{2} \frac{\ln\left(\frac{\sqrt{(4\xi_2^2+1)}+2\alpha-1}{\sqrt{(4\xi_1^2+1)}+2\alpha-1}\right)}{\ln\gamma} + \frac{\ln\left(\frac{\sqrt{4\xi_2^2+1}+1)}{\sqrt{4\xi_1^2+1}+1)}\right)}{2\ln\gamma} \qquad (18)$$

where $\xi = C/\phi x$

On the other hand Kobatake (18) the mass transference number of Co-ions in the negatively charged membrane is given by

$$T_{app} = 1 - \alpha\frac{\sqrt{(4\xi^2+1)}+1}{\sqrt{(4\xi^2+1)}+(2\alpha-1)} \qquad (19)$$

where ξ and α stands for relative concentration as defined by $C/\phi x$ and $\frac{u}{u+v}$ respectively.

Using certain eqns. proposed by Kobatake and Kamo (17) for the activity coefficients, mobilities of small ions in the membrane phase and the equilibrium condition for electrical neutrality. Practically, the difference between T_- and T_{app} was found to be less than 2% within the wide range of salt concentration (18). If T_- is replaced by T_{app} and C by $\frac{C_1 + C_2}{2}$ and eqn. (19) is rearranged, the permselectivity (Ps) is obtained by the following expression.

$$\frac{1}{(4\xi^2 + 1)^{1/2}} = \frac{\left[1 - T_{app} - \alpha\right]}{\left[\alpha - (2\alpha - 1)(1 - T_{app})\right]} \equiv Ps \qquad \text{--------} \quad (20)$$

Here Ps is a measure of permselectivity of the membrane electrolyte system.

In the case where a positively charged membrane is concerned Kobatake et al. (18) have derived another eqn. (21) to define permselectivity (Ps) for a +vely charged membrane

$$Ps = \frac{\left[T_{app} - (1-\alpha)\right]}{\left[1 - \alpha - (1-2\alpha)\, T_{app}\right]} \qquad \text{----------} \quad (21)$$

Here Ps is a measure of permselectivity for positively charged membrane.

Nagasawa et al. (20) derived an eqn. for membrane potential existing across a charged membrane. The total membrane potential $\Delta\phi$ was considered as the sum of diffusion potential $\Delta\phi_d$

inside the membrane and the electrostatic potential difference $\Delta\phi_e$ between the membrane surface and the electrolyte solutions on both sides of the membrane. The diffusion potential $\Delta\phi_d$ was obtained by integrating the basic flow equation for diffusion (20) while the electrostatic, potential difference was calculated from Donnan's theory, i.e.,

$$\Delta\phi = \Delta\phi_d + \Delta\phi_e \qquad \text{----------(22a)}$$

where

$$-\Delta\phi_d = -\int_1^2 \left(\frac{Jo}{F\bar{C}_o}\right) \frac{\phi x}{(\bar{C}_- + \phi x)\, u_+ + \bar{C}_-\, u_-}\, dx +$$

$$\frac{RT}{F} \int_1^2 \frac{(\bar{C}_- + \phi x)\, u_+}{(\bar{C}_- + \phi x)\, u_+ + \bar{C}_- u_-}\, d\ln\bar{a}_+$$

$$-\frac{RT}{F} \int_1^2 \frac{\bar{C}_- u_-}{(\bar{C}_- + \phi x)\, u_+ + \bar{C}_- u_-}\, d\ln\bar{a}_- \qquad \text{----------(22b)}$$

$$\text{and } -\Delta\phi_e = -\frac{RT}{F} \ln \left(\frac{\bar{a}_{1-} - a_{2-}}{a_{1-} - \bar{a}_{2-}}\right) \qquad \text{---------(22c)}$$

where a_1 and a_2 are the activities of the electrolytes on two sides of the membrane. Jo is the flow of electrolyte in absence of the external electric field. By integrating equation (22) and putting the limit of high electrolyte concentrations across the membrane the following equation for membrane potential is obtained.

$$-\Delta\phi = \frac{RT}{F}\left(\frac{\phi x}{2}\right)\left(\frac{\gamma-1}{\gamma}\right)\left(\frac{1}{C_1}\right) + \frac{RT}{F}\left(\frac{u_+ - u_-}{u_+ + u_-}\right) x$$

$$\left[\frac{1 - \dfrac{\phi X J_o}{RT\bar{C}_o\ (u_+ - u_-)\ K}}{1 - \dfrac{\phi X J_o}{2RT\bar{C}_o u_- K}}\right] \ln\gamma$$

$$+ \left(\frac{RT}{2F}\right)\left(\frac{\phi x}{u_+ u_-}\right)\left(\frac{J_o}{RT\bar{C}_o\ K}\right)^2 \frac{\left[1 - \dfrac{\phi X J_o\ (u_+ + u_-)}{4RT\ \bar{C}_o\ u\ u_-\ K}\right]}{\left[1 - \dfrac{\phi X J_o}{2RT\bar{C}_o\ u_- K}\right]}$$

$$(\gamma-1)\ C_1 \text{ ----------(23)}$$

At high electrolyte concentration equation (23) was put in the following approximate form,

$$-\Delta\phi = \frac{RT}{F}\left(\frac{\gamma-1}{\gamma}\right)\left(\frac{\phi x}{2}\right)\frac{1}{C_1} + \text{ ----------(24)}$$

Equation (24) predicts a linear relationship between $\Delta\phi$ and $\frac{1}{C_1}$ from which ϕx can be calculated.

MATERIALS AND METHODS

EXPERIMENTAL

The pericardial membrane was removed just after experimental animal (i.e. buffalo and aged between 18-24 months) was slain. This was accomplished with the help of scissors. The membrane was immediately submersed in ice-cold ringer solution of about 7.4 ± 0.2 pH (109-110) for preservation of the membrane tissues. The constituents used for Ringer solution (mgms/1) were as follows: NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, Glucose 1.0, and $NaHCO_3$ 0.15.

APPARATUS AND EXPERIMENTAL METHOD:

The diagram of the apparatus used for the measurement of membrane potential is shown in fig. C. It consists of two half cells. The vertical female joints Y and Y' attached to each half cell provide for introducing the electrolyte solution and the saturated calomel electrodes (S.C.E.) X_1 and X_2. The cell is divided into two symmetrical compartments by a water-tight membrane which was placed between the brims of these two cell parts. Both the solutions were stirred vigorously.

MEASUREMENT OF MEMBRANE POTENTIAL

The membrane was washed three times with deionized water for the removal of ringer solution before observing the

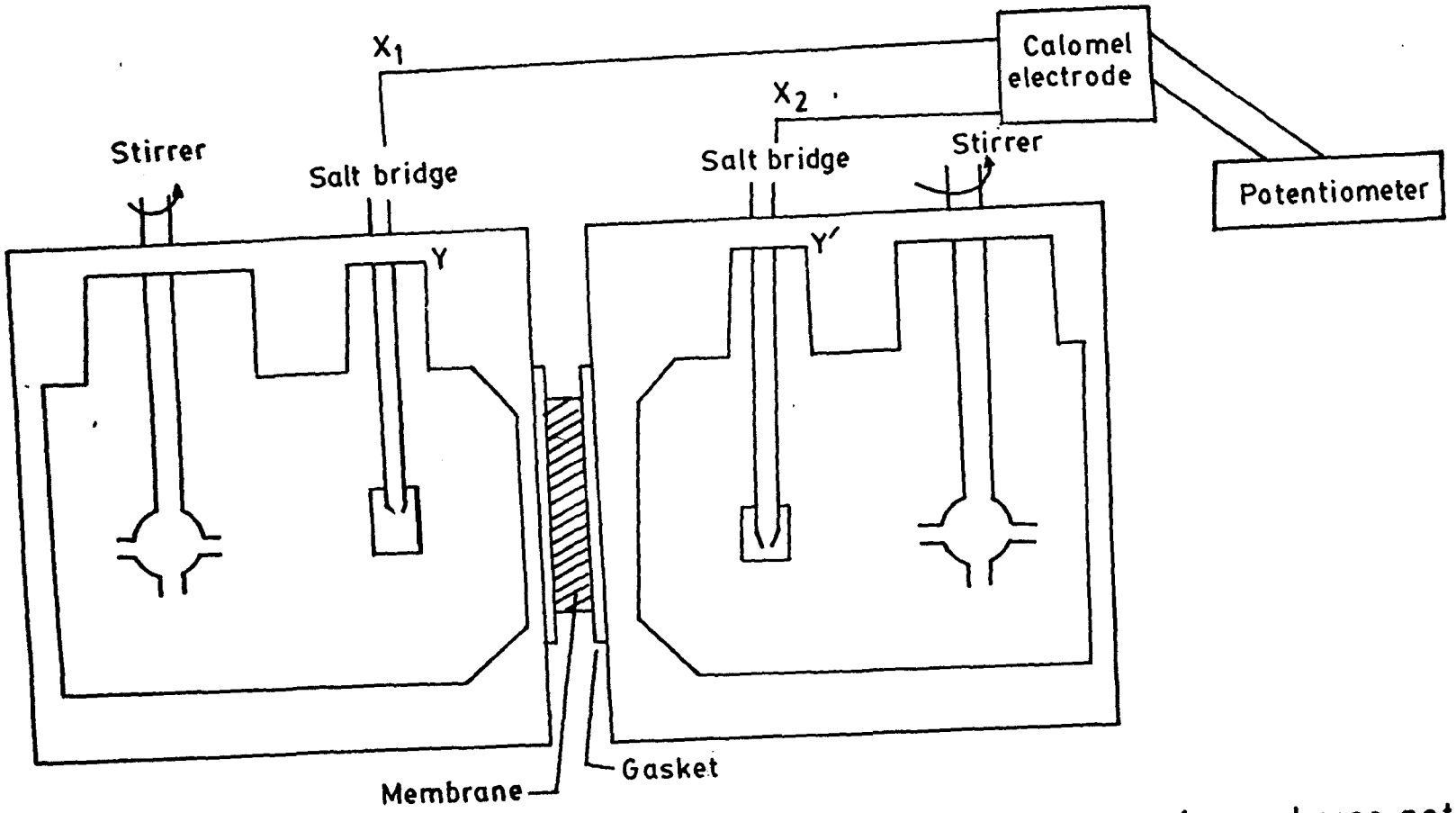


Fig.C: Schematic diagrame of cell used for the measurement of membrane potential.

membrane potential. The potential developed by setting up a concentration cell of the type described by Sollner and Gregor (111), Marshall and Ayers (105) and Michaelis (102) was taken as a measure of membrane potential by using a pye-precision vernier potentiometer (No. 7568). The whole cell assembly was immersed in a water thermostate maintained at 25°C with constant stirring. The various salt solutions e.g. NaCl, NaF Kcl, KF, NH_4Cl and some trace metal salts were prepared from analytical grade (B.D.H.) reagents by the use of deionized water.

Electrochemical cell of the type

S.C.E.	Solution	Membrane	Solution	S.C.E.
	C_1		C_2	

$C_2 = 10C_1$

were used for measuring membrane potentials.

The same electrolyte with different concentration was used on both sides of the membrane. The dilute side is taken as negative. Fresh pericardial membrane was used for potential measurements. The experiments were repeated with fresh solution of electrolyte and the maximum potential attained was recorded.

The membrane potential data obtained with each of the pericardium using various 1:1 and 2:1 electrolytes, are plotted as a function of log $\frac{C_1 + C_2}{2}$ while the ratio $= \frac{C_2}{C_1}$ fixed at 10. These flots are shown in figs. (1 and 2). The membrane potential data obtained are given in the tables (1-7) for univalent and divalent electrolytes.

TABLE - 1

Pericordial membrane potential at different concentrations of Sodium chloride at 25°C

S.No.	Time (minutes)	Potentials (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.05/0.005M	0.02/0.002M	0.01/0.001M
1	0	17.25	24.49	30.30	35.30	36.63	29.48	18.35
2	5	15.58	26.36	35.33	42.31	50.31	48.51	52.25
3	10	14.27	26.38	35.69	42.28	51.29	54.37	58.33
4	15	12.50	24.25	36.29	44.26	49.58	51.57	58.36
5	20	11.12	22.38	35.35	43.27	49.30	52.33	53.46
6	25	11.19	20.46	35.16	43.21	51.44	54.32	53.12
7	30	9.20	19.32	35.16	42.67	47.22	55.37	52.39
Maximum membrane potential		17.25	26.38	36.29	44.26	51.44	55.37	58.36

All observed membrane potentials are in negative.

TABLE - 2

Pericardial membrane potential at different concentrations of sodium fluoride at 25°C

S.No.	Time (minutes)	Potentials (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.05/0.005M	0.02/0.002M	0.01/0.001M
1	0	11.32	17.28	24.32	29.34	32.37	40.40	16.71
2	5	10.36	17.46	25.32	29.63	32.64	41.45	32.36
3	10	10.38	18.40	22.85	27.88	30.98	35.72	33.28
4	15	10.61	17.20	22.06	22.21	32.30	32.30	35.32
5	20	10.51	16.65	24.20	18.18	35.29	37.27	39.29
6	25	10.16	16.31	24.61	24.08	33.37	38.76	46.30
7	30	7.07	17.36	24.23	29.28	43.39	41.38	36.55
Maximum membrane potential		11.32	18.40	25.32	29.63	35.29	41.45	46.30

All observed membrane potentials are in negative.

TABLE -

Pericardial membrane potential at different concentrations of potassium chloride at 25°C

S.No.	Time (minutes)	Potential (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.05/0.005M	0.02/0.002M	0.01/0.001
1	0	6.92	8.94	10.78	10.20	12.11	9.20	7.01
2	5	9.28	9.73	11.22	9.15	15.23	12.35	16.14
3	10	8.55	6.11	11.35	9.09	12.13	16.29	10.30
4	15	9.89	5.40	11.98	11.33	13.21	16.32	9.28
5	20	7.49	10.72	8.32	12.32	12.32	15.98	7.09
6	25	5.79	10.54	7.14	10.24	11.18	14.38	4.15
7	30	4.82	7.60	7.13	8.27	12.27	15.24	4.27
Maximum membrane Potential		9.89	10.72	11.98	12.32	15.23	16.32	16.14

All observed membrane potentials are in negative.

TABLE - 4

Pericardial membrane potential at different concentrations of Potassium fluoride at 25°C

S.No.	Time (minutes)	Potential (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.05/0.005M	0.02/0.002M	0.01/0.001M
1	0	5.49	9.24	11.25	14.25	13.35	17.49	9.29
2	5	3.28	12.33	20.15	26.36	26.36	28.37	20.34
3	10	5.26	9.21	21.28	20.29	27.28	32.54	27.28
4	15	4.15	8.20	20.22	17.25	32.04	34.32	38.26
5	20	4.11	7.33	18.24	17.23	29.21	34.49	34.49
6	25	4.05	7.10	17.14	15.04	29.60	29.75	33.51
7	30	3.28	6.15	16.17	15.15	31.36	28.17	34.22
Maximum membrane potential		5.49	12.33	21.28	26.36	32.04	34.49	38.26

All observed membrane potentials are in negative.

TABLE - 5

Pericardial membrane potential at different concentrations of Ammonium chloride at 25°C

S.No.	Time (minutes)	Potential (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.05/0.005M	0.02/0.002M	0.01/0.001M
1	0	3.76	8.32	16.37	15.45	21.29	23.33	18.47
2	5	4.15	9.35	18.26	20.60	26.67	26.27	21.39
3	10	4.20	9.65	18.29	22.08	27.70	27.48	24.61
4	15	3.11	9.22	19.31	23.12	26.78	25.29	25.13
5	20	3.64	8.38	17.90	24.45	27.04	25.72	23.42
6	25	3.58	7.75	17.28	22.44	25.79	26.38	23.77
7	30	3.04	4.10	16.21	22.82	26.27	26.59	23.40
Maximum membrane potential		4.20	9.65	19.31	24.45	27.70	27.48	25.13

All observed membrane potentials are in negative.

TABLE - 6

Pericardial membrane Potential at different concentrations of Nickel Chloride at 25°C

S.No.	Time (minutes)	Potential (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.5/0.005M	0.02/0.002M	0.01/0.001M
1	0	22.02	23.34	30.02	33.31	39.33	45.45	37.35
2	5	22.28	19.43	29.66	32.96	35.30	43.29	41.36
3	10	22.46	16.32	29.50	32.12	36.13	44.38	40.60
4	15	22.56	15.75	27.11	33.06	38.31	43.34	40.38
5	20	22.40	14.77	20.78	31.72	39.20	43.36	40.09
6	25	21.64	14.22	19.44	31.77	39.09	43.17	41.32
7	30	21.17	14.13	18.44	33.13	38.07	43.00	43.93
Maximum membrane potential		22.56	23.34	30.02	33.31	39.33	45.45	43.93

All observed membrane potentials are in negative.

TABLE - 7

Pericardial membrane potential at different concentrations of cobalt chloride at 25°C

S.No.	Time (minutes)	Potential (mV)						
		1/0.1M	0.5/0.05M	0.2/0.02M	0.1/0.01M	0.05/0.005M	0.02/0.002M	0.01/0.001M
1	0	19.98	22.10	26.18	28.23	29.04	33.29	35.85
2	5	17.72	22.18	27.11	29.13	30.35	37.18	39.28
3	10	15.01	15.78	26.08	27.30	30.46	38.24	42.35
4	15	19.00	20.62	24.02	23.13	30.31	38.16	41.38
5	20	14.20	16.06	25.25	18.16	28.07	38.44	40.20
6	25	12.31	10.90	24.00	25.84	25.05	38.00	39.30
7	30	17.45	12.82	21.00	23.84	29.21	37.44	40.05
Maximum membrane potential		19.98	22.18	27.11	29.13	30.46	38.44	42.35

All observed membrane potentials are in negative.

RESULTS AND DISCUSSION

The values of membrane potential ($\Delta\phi$) measured across pericardial membrane separating different concentrations of 1:1 and 2:1 electrolytes (chlorides of sodium, potassium, ammonium, Nickel, cobalt and fluorides of sodium and potassium) are given in tables 1-7 and are also plotted as a function of $\log \frac{C_1 + C_2}{2}$ with the ratio of $\gamma = C_2/C_1$ fixed at 10. These are shown in figures 1 and 2.

The values of membrane potential were found to be negative in all the electrolyte concentrations (concentrated solution side taken as +ve). This means that this pericardial membrane is positively charged (anion selective) and selectivity increases with dilution Siddiqui et al. (112).

For the evaluation of the membrane fixed charge density by potentiometric method, we have employed various approaches viz; Nagasawa et al. (20) and different methods of Kobatake et al. (17, 18, 99) based on the thermodynamics of irreversible process.

Starting with the basic flow equations provided by the thermodynamics of irreversible processes (already described in the begining), Kobatake (99) derived the following final expression for the membrane potential $\Delta\phi$ which arises between two solutions of 1:1 electrolyte of different concentrations C_1 and C_2 that are separated by a membrane: Where C_1 concentration is less than the C_2 ($C_1 < C_2$).

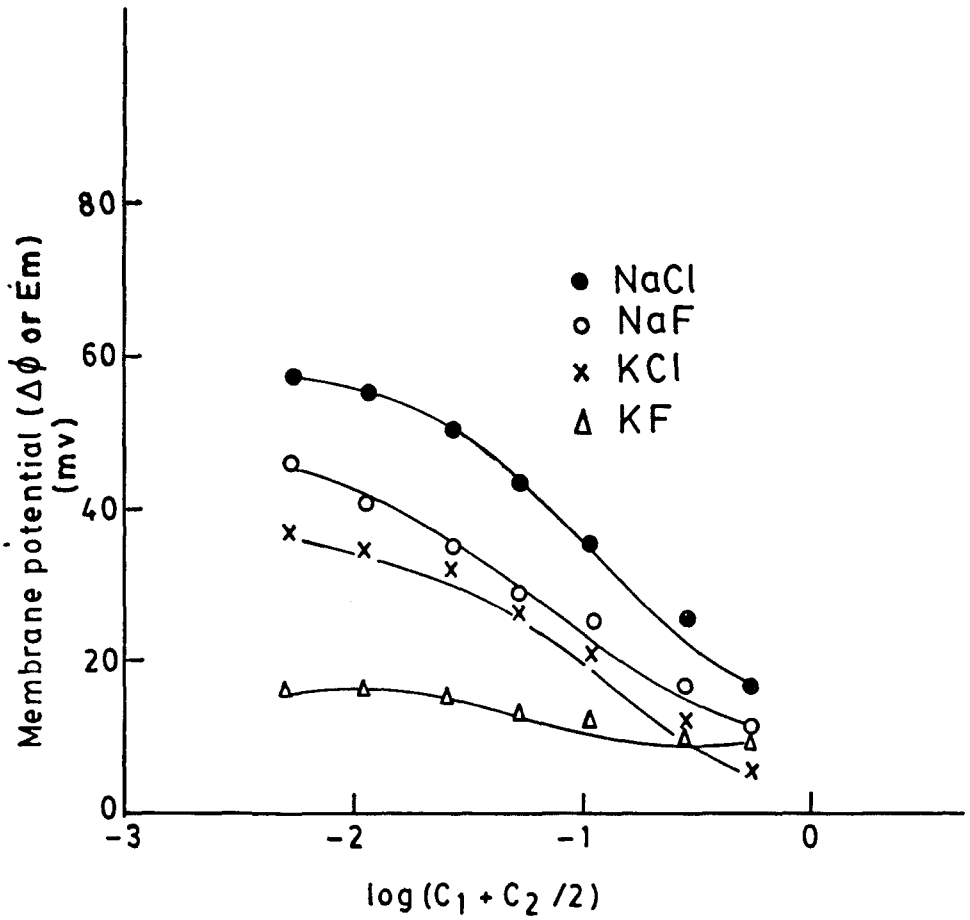


Fig.1: Plots of observed potentials against log $C_1 + C_2/2$ for various electrolytes with pericardium

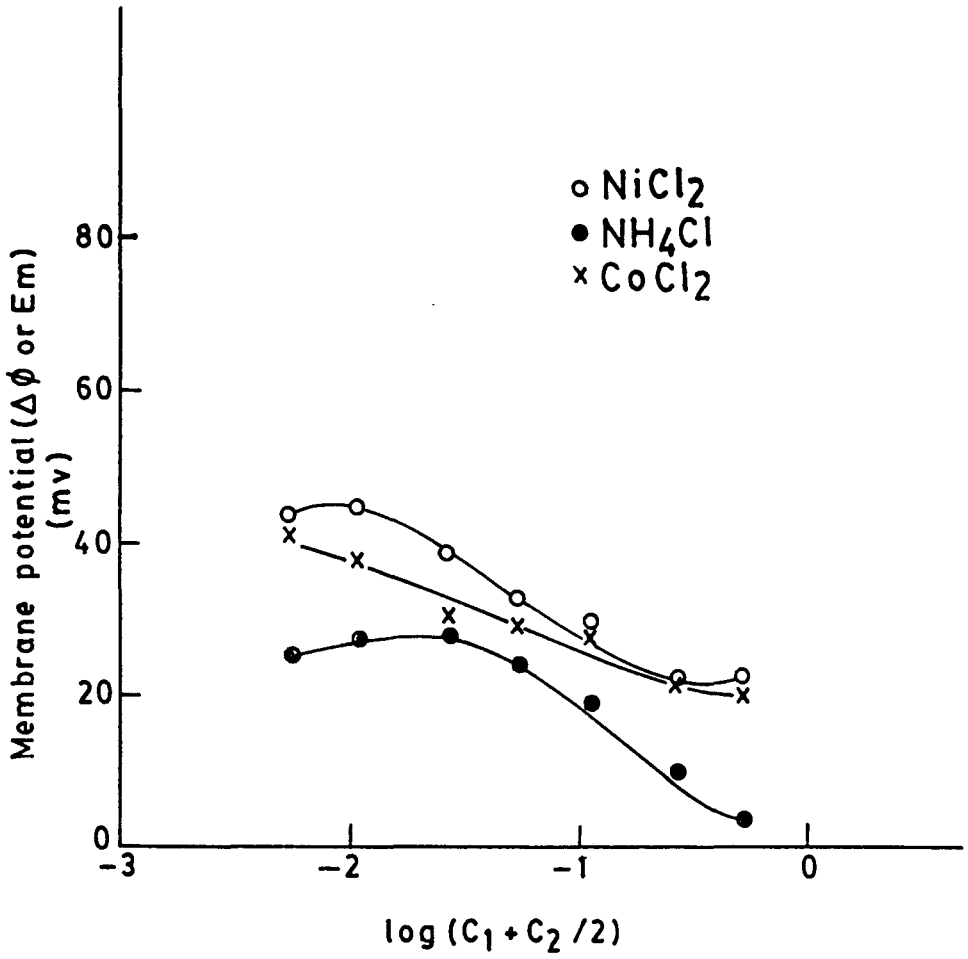


Fig.2: Plots of observed potentials against log $C_1 + C_2/2$ for various electrolytes with pericardium

$$\Delta\phi = -\left(\frac{RT}{F}\right)\left[\frac{1}{\beta}\ln\frac{C_2}{C_1} - \left(1 + \frac{1}{\beta} - 2\alpha\right)\ln\left(\frac{C_2 + \alpha\beta\theta}{C_1 + \alpha\beta\theta}\right)\right] \quad \ldots\ldots(1)$$

where $\alpha = u/(u+v)$ $\ldots\ldots(1a)$

and $\beta = 1 + \left(\frac{KF\theta}{u}\right)$ $\ldots\ldots(1b)$

where u and v are the molar mobilities of +ve and -ve ions; respectively, defined in terms of the mass fixed frame of reference, K is a constant which is considered to depend on the viscosity of the solution and structural details of the polymer net-work of which the membrane is composed, θ is the charge density and F is the Faraday constant. These parameters have been assumed to be independent of salt concentration C_2 and C_1.

For the analysis of data, equation (1) can be used under two sets of condition namely (a) in the dilute range and (b) in the concentrated range and hence the two limiting forms of the above equations have been derived,

(a) When concentration C_2 becomes sufficiently small, (external dilute range) equation (1) can be expanded to give

$$|\Delta\phi_r| = \frac{1}{\beta}\ln\gamma - \left(\frac{\gamma-1}{\alpha\beta\gamma}\right)\left(1 + \frac{1}{\beta} - 2\alpha\right)\frac{C_2}{\theta} + \ldots\ldots\ldots(2)$$

where $|\Delta\phi_r|$ is the absolute value of reduced potential defined by

$$|\Delta\phi_r| = F\Delta\phi \,/\, RT \quad \ldots\ldots\ldots(3)$$

and $\gamma = C_2/C_1$

Equation (2) indicates that the value of β and a relation between α and θ can be observed by evaluating the intercepts and initial slope of a plot for $\Delta\phi r$ against C_2. Figure 3 illustrate plots for $\Delta\phi r$ against C_2 in the region of low concentration for five monovalent electrolytes with pericardial membrane observed in this study. The value of intercept is equal to $\frac{1}{\beta} \ln\gamma$, from which β may be evaluated. The various values of β obtained with pericardium are given in table 8.

(b) It is well known experimentally that at fixed γ, the inverse of an apparent transference number, i.e. t_{app}, for this membrane is proportional to the inverse of concentration C_2, when the salt concentration is high. Here t_{app} is defined by the relation

$$|\Delta\phi r| = (1-2\, t_{app}) \ln\gamma \qquad \ldots\ldots\ldots\ldots(4)$$

The values of transport number (t_{app}) calculated from observed membrane potentials using equation (4) are given in table (9). Kobatake et al. (99) have proposed a simple method using the following approximate equation for the diffusive contribution to the emf. of cell with transport. On substituting equation (3) into equation (4), the following relation between $\Delta\phi$ and t_{app} is obtained.

$$\frac{F\Delta\phi}{RT} = (1-2\, t_{app}) \ln\gamma \qquad \ldots\ldots\ldots\ldots(5)$$

where t_{app} is the apparent transference number in the membrane phase.

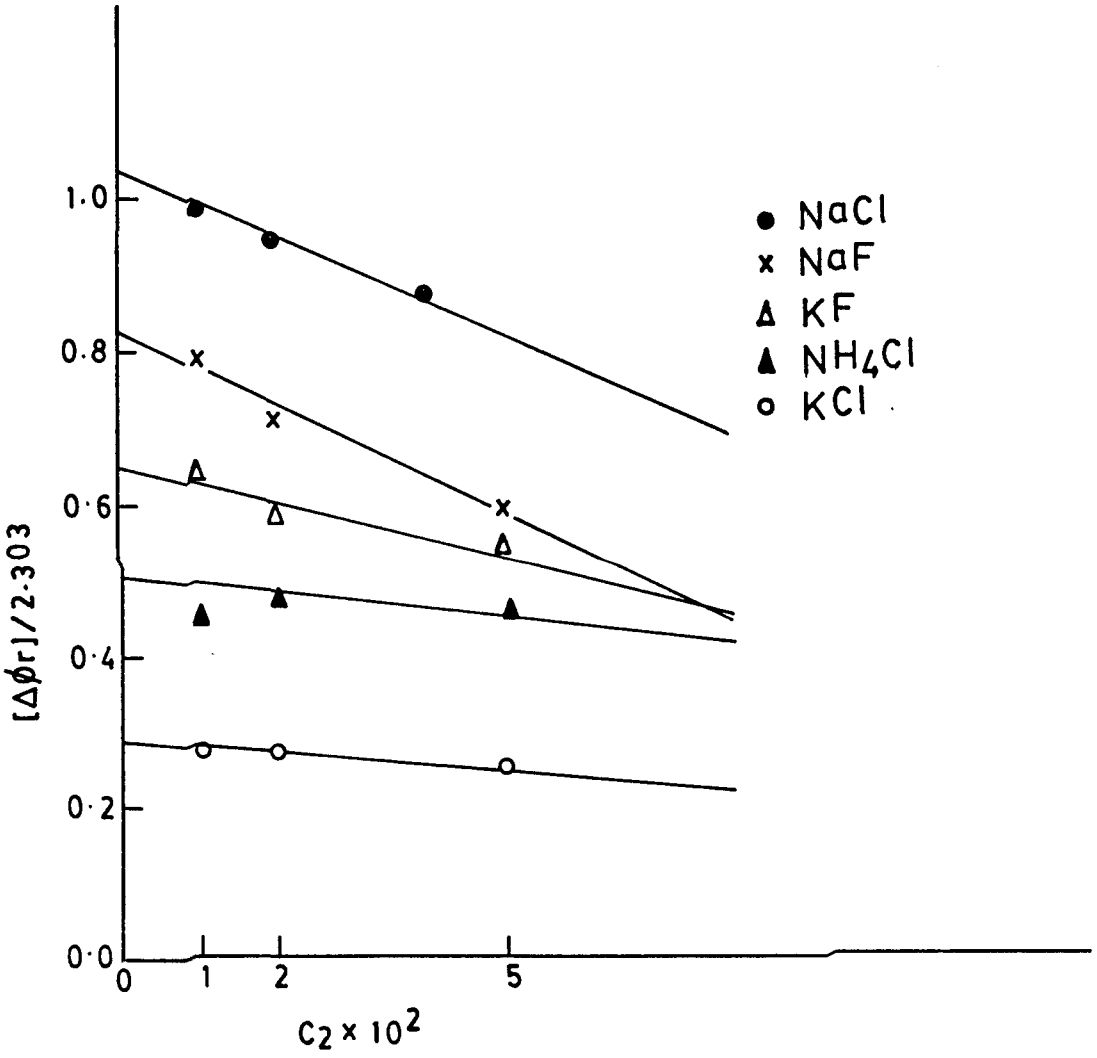


Fig.3: Plots of $[\Delta \phi r]/2.303$ vs $C^2 \times 10^2$ for various electrolytes with pericardium

TABLE - 8

The derived values of Parameter α and β for various electrolytes with Pericardium at γ = 10.

S. No.	Electrolyte	α	β
1	NaCl	0.56	0.97
2	NaF	0.55	1.22
3	KCl	0.59	3.51
4	KF	0.49	1.55
5	NH4Cl	0.44	2.00

TABLE - 9

Transference number (t_{app}) derived from the observed membrane potentials at various electrolyte concentrations at Υ =10 for pericardium.

S.No.	Concentration C_2/C_1 (mol /l)	Electrolytes				
		NaCl	NaF	KCl	KF	NAuCl
1	$1/1x10^{-1}$	0.354	0.404	0.380	0.453	0.464
2	$5x10^{-1}/5x10^{-2}$	0.277	0.344	0.395	0.395	0.418
3	$2x10^{-1}/2x10^{-2}$	0.193	0.286	0.354	0.320	0.336
4	$1x10^{-1}/1x10^{-2}$	0.126	0.249	0.396	0.277	0.293
5	$5x10^{-2}/5x10^{-3}$	0.065	0.202	0.371	0.229	0.266
6	$2x10^{-2}/2x10^{-3}$	0.032	0.149	0.362	0.208	0.268
7	$1x10^{-2}/1x10^{-3}$	0.007	0.109	0.364	0.176	0.288

Substituting for $\Delta\phi$ from equation (1) and expanding the resulting expression for $1/t_{app}$ in powers of $1/C_2$, gives

$$1/t_{app} = \frac{1}{(1-\alpha)} + \frac{(1+\beta-2\alpha\beta)\ (\gamma-1)}{2(\ 1-\alpha)^2\ \ln\gamma}\ (\ \frac{e}{C_2}\ ) + \ldots\ldots\ldots\ldots\ldots(6)$$

This indicates that the intercept for a plot of $1/t_{app}$ against $1/C_2$ at fixed γ allows the value of α to be determined. The value of the intercept is equal to $1/1-\alpha$, from which α may be evaluated. Figures 4 and 5 illustrate plots for $1/t_{app}$ against $1/C_2$ for various electrolytes with pericardium.

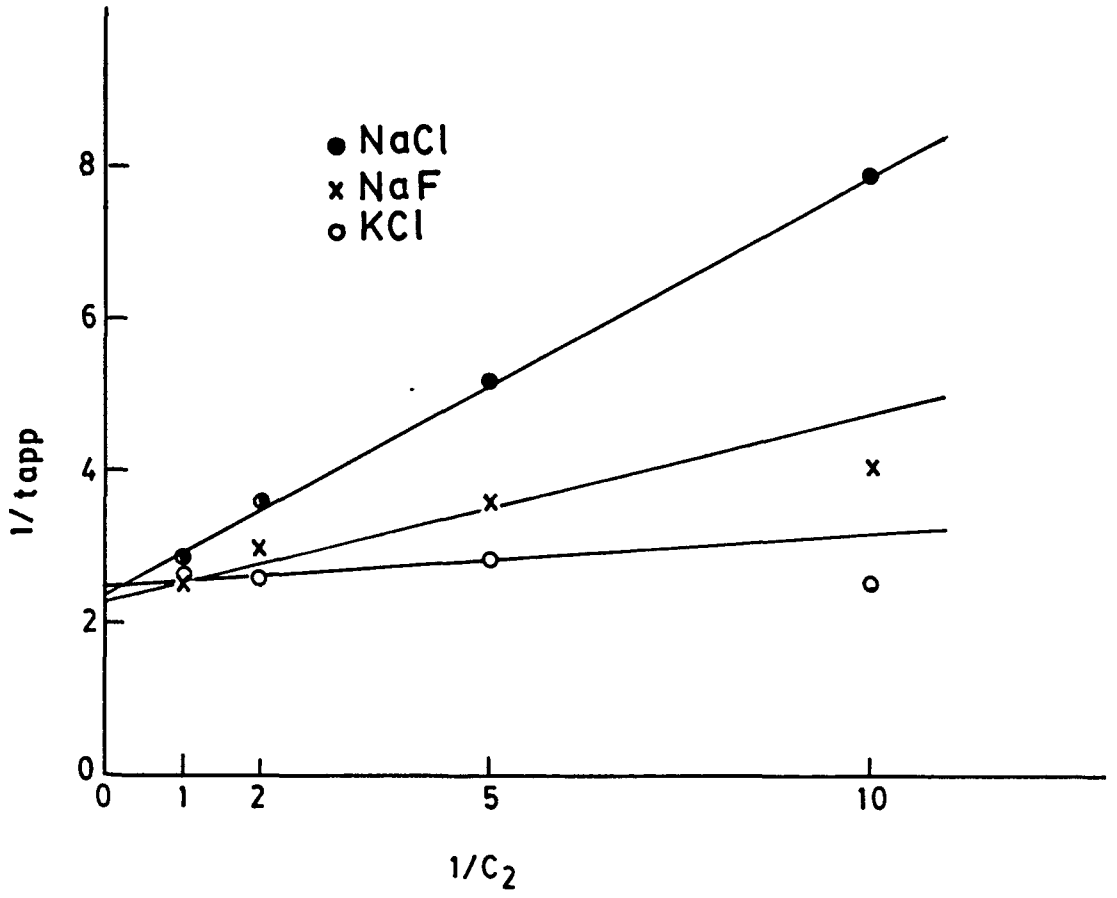
The values of α thus evaluated for pericardial membrane are given in table 8.

For the evaluation of charge density e, there are also two limiting cases:

a) In the dilute range the charge density (e_d), may be evaluated with the slope of figure 4 and 5 equated with

$$\frac{\gamma-1}{\alpha\beta\gamma}\ (1 + \frac{1}{\beta} - 2\alpha)\ \ \frac{1}{e}$$

which is equated with the graphical values of the slope of figure 3 for pericardial membrane. The predetermined values of α and β for pericardium are substituted in the expression and thus e is evaluated. This value of e is designated as e_d. The values of charge density e_d evaluated in the dilute range in this manner are listed in the table 11.



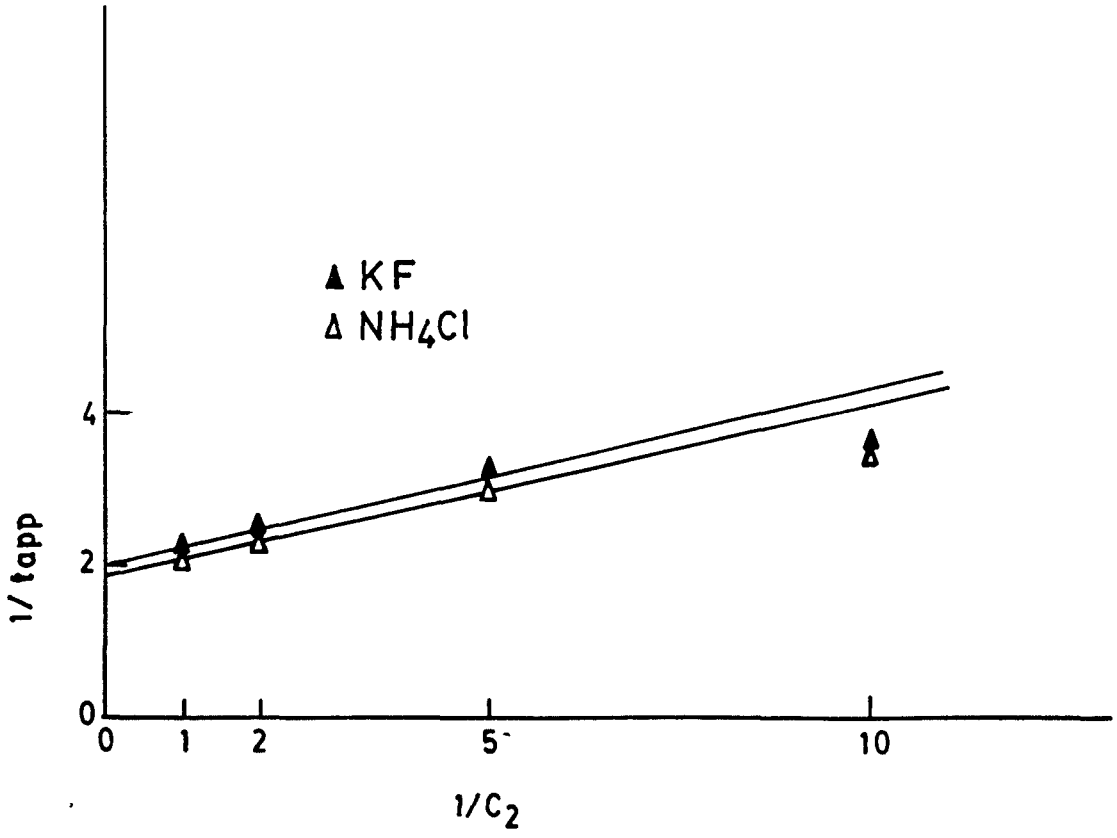


Fig.4&5: Plots of 1/tapp vs 1/C2 for various electrolytes with pericardium

b) In the concentrated range using equation (6), the slope is given by

$$\frac{(1+\beta-2\alpha\beta)(\gamma-1)\alpha}{2(1-\alpha)^2 \ln\gamma} e$$

The graphical value of the slope determined from figure 4 and 5 for pericardial membrane is equated with the above expression. The values of α and β determined already are substituted and thus the value of fixed charge density e is evaluated for pericardium. The value of e is designated as e_p. The values of charge density evaluated in this manner are given in the table 11.

In the present investigation, the magnitude of the values of e (i.e. e_d and e_c) obtained from the opposite limits agree with each other and it can safely be concluded that the Kobatake's equation is applicable to this membrane. Also the values of α in Kobatake's treatment is defined by u/(u+v) where u and v stand for the mobilities of cation and anion respectively in free solution. The values of α thus obtained for various electrolytes with pericardium are given in table 8. It is evident that the constancy of the stoichiometric fixed charge densities of the investigated membranes which is the basic assumption of the TMS theory (19, 90-92, 115) and its revised form given by Schlogl (113,114) and Kobatake et al. (99) are applicable to pericardium.

Comparison can be made between theoretical and experimental data, and the applicability of Kobatake et al. (99) equation to the membranes under study can be tested by the following analytical technique suggested by Kobatake and coworkers (99). Equation (1) can be rewritten as

$$\frac{\gamma - e^{q}}{e^{q} - 1} = Z \qquad \ldots\ldots\ldots\ldots (7)$$

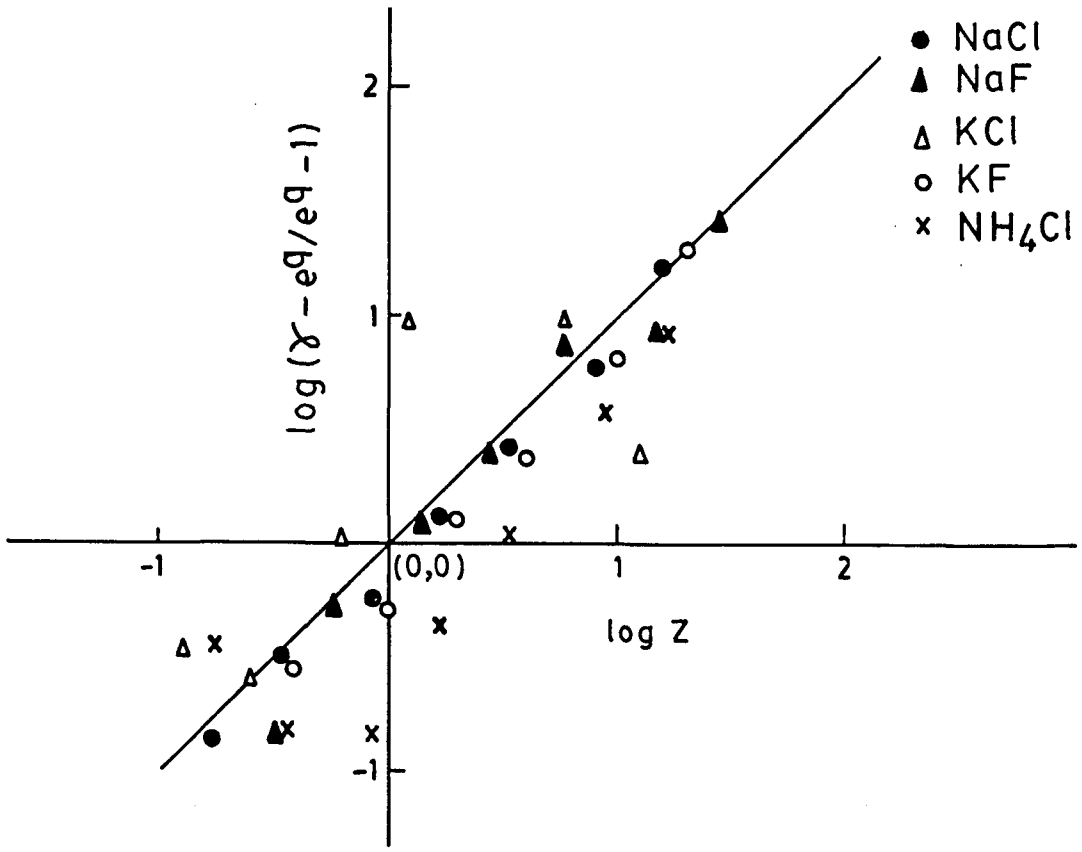
where q and Z are defined by

$$q = \left[\Delta\phi r + (1-2\alpha)\ln\gamma\right] \Big/ \left[\left(\frac{1}{\beta}\right) + (1-2\alpha)\right] \qquad \ldots\ldots\ldots (8)$$

$$Z = \frac{c_2}{\alpha\beta e} \qquad \ldots\ldots\ldots (9)$$

Thus, if equation (1) is valid, the values of $\log\left(\frac{\gamma - e^{q}}{e^{q} - 1}\right)$ calculated from the measured $\Delta\phi$ with the predetermined α, β and e and the given value of γ must fall on a straight line, which has a unit slope and passes the coordinate origin when plotted against log Z. This behaviour must be valid irrespective of the value of γ and the kind of membrane-electrolyte system. Figure 6 demonstrates that this theoretical prediction of membrane potential equation is borne out quite satisfactorily by our experimental results on pericardial membrane.

Kobatake and Kamo (17) derived another equation (10) for the membrane potential using a different set of assumptions,



ig.6: Plot of $\log(\gamma - e^q/e^q - 1)$ vs log z for pericardium in contact with various 1:1 electrolyte solutions

namely (i) the contribution of mass movement is negligible and (ii) small ion do not behave ideally in the charged membrane.

$$\Delta\phi = -\,RT/F \ln \frac{C_2}{C_1} + (2\alpha-1) \ln \frac{\sqrt{4C_2^2 + \phi^2X^2} + (2\alpha-1)\phi X}{\sqrt{4C_1^2 + \phi^2X^2} + (2\alpha-1)\phi X}$$

$$-\ln \frac{\sqrt{4C_2^2 + \phi^2X^2} + \phi X}{\sqrt{4C_1^2 + \phi^2X^2} + \phi X} \qquad \ldots\ldots\ldots(10)$$

where ϕ is a characteristic factor of the membrane electrolyte pair. The product ϕX is termed as the thermodynamically effective fixed charge density of a membrane. Equation 10 has the same functional form as that given by the TMS theory for membrane potential $\Delta\phi$ except that the thermodynamically effective fixed charge density ϕX of the membrane is used in place of stoichiometric fixed charge density. Equation 10 reduces the TMS membrane potential for $\phi = 1$. Since it was some what troublesome to evaluate the effective fixed charge density ϕX at an arbitrary external electrolyte concentration from the observed membrane and Kobatake and Kamo (17) have proposed a simple method using the following approximate equation for the diffusive contribution to the emf of a cell with transport.

$$\Delta\phi = -\,RT/F\,(1-2\,t_{app})\ln C_2/C_1 \qquad \ldots\ldots\ldots(11)$$

where t_{app} is the apparent transference number in the membrane phase.

This modification of TMS theory by Kobatake and Kamo (17) has been adopted by us for analysis of our results.

A general method for characterization of membranes in terms of Permselectivity which is applicable to any system irrespective of ion species has been developed by Kobatake (18). With the same assumption Kobatake derived equation (12) and (13) for the measurements of Permselectivity (Ps) for both negatively and positively charged membranes respectively.

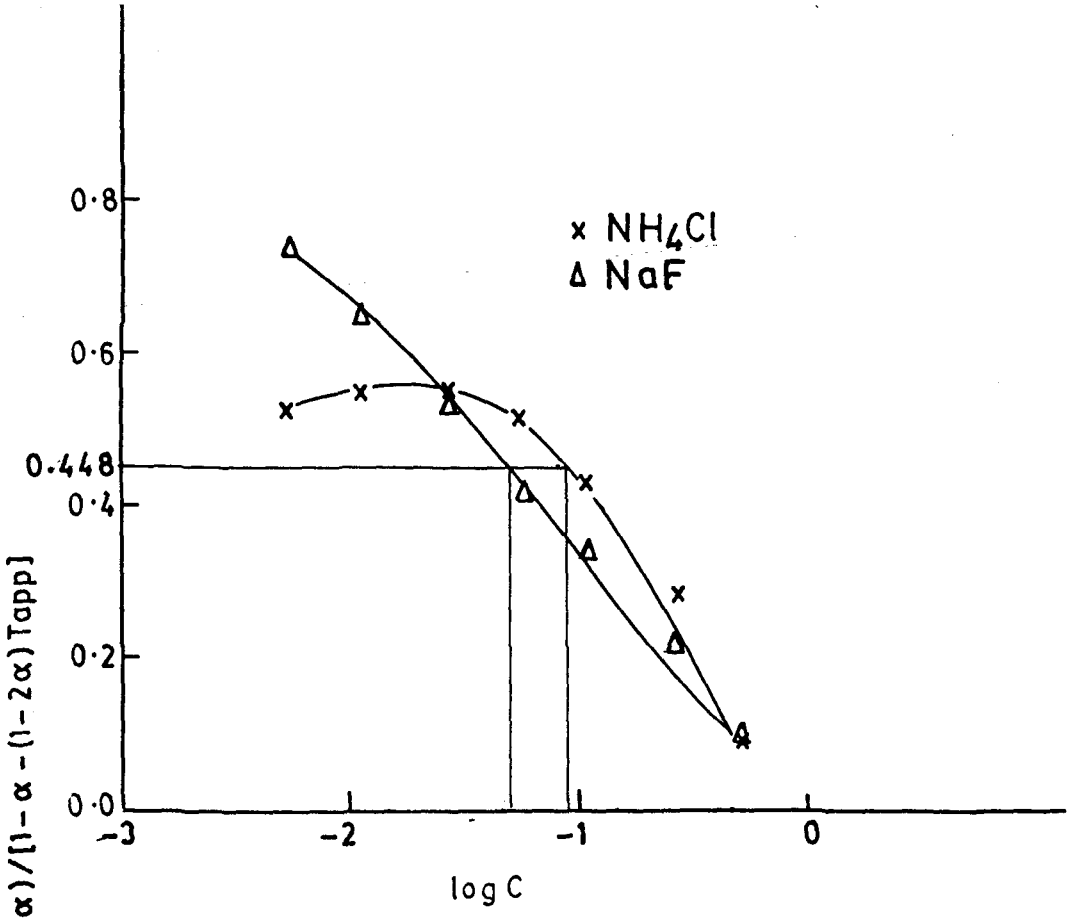
$$\frac{1}{(4\xi^2+1)^{\frac{1}{2}}} = \frac{\left[1 - T_{app} - \alpha\right]}{\left[\alpha - (2\alpha - 1)(1 - T_{app})\right]} \equiv Ps \quad \text{.....(12)}$$

$$Ps = \frac{\left[T_{app} - (1 - \alpha)\right]}{\left[1 - \alpha - (1 - 2\alpha)T_{app}\right]} \quad \text{-.....(13)}$$

Ps is a measure of permselectivity of membrane electrolyte systems. The values of Ps takes between Zero and unity depending on the external salt concentrations for the given system of membrane and electrolyte pair. Ps can be calculated from the data of membrane potential, while left hand side of equation (12 and 13), is a function of the relative concentration $\xi = \frac{C}{\phi X}$ or $\frac{C_1 + C_2}{2\phi X}$. Thus, the value of the right hand side should be independent of the mobilities of ion species involved. Equation 12 and 13 implies that the plot of Ps Vs $(1 + 4\xi^2)^{-\frac{1}{2}}$ should give a straight line of unit slope. The various

values of Permselectivity were calculated by substituting the value of α and t_{app} from table 8 and 9 for pericardium. The values of Ps were given in table 10. The other method for the evaluation of charge density based on Permselectivity was developed by Kobatake (18) and is also used here. The calculated values of Ps on pericardial membrane were plotted against log $\frac{C_1 + C_2}{2}$. The curves with various electrolytes were obtained and shown in figures 7,8 for pericardium when the average concentration C i.e. $\frac{C_1 + C_2}{2}$ becomes equal to the effective fixed charge density $\emptyset X$, the value of ξ becomes unity i.e. $\frac{C}{\emptyset X} = 1$, Substituting this value of $\xi = 1$ into $Ps = (1 + 4\xi^2)^{-\frac{1}{2}}$, the value of Ps = 0.448 is obtained. At this particular value of 0.448 the corresponding concentration was obtained from the curve drawn between Ps Vs. log C. It is apparant from figure that this value of concentration should be equal to fixed charge density for with various electrolytes, by using figures 7 and 8. The values of charge density obtained with various electrolytes are given in table 11. Plots of Ps Vs. $(1+4\xi^2)^{-\frac{1}{2}}$ drawn with Pericardial membrane by using various electrolytes and are shown in figure 9. It is evident that the line nearly passes through the origin with unit slope, thereby confirming the applicability of Kobatake's equation to this membrane.

Kobatake and Kamo (18) formulated equation (14) for evaluation of charge density. Using this equation we plotted



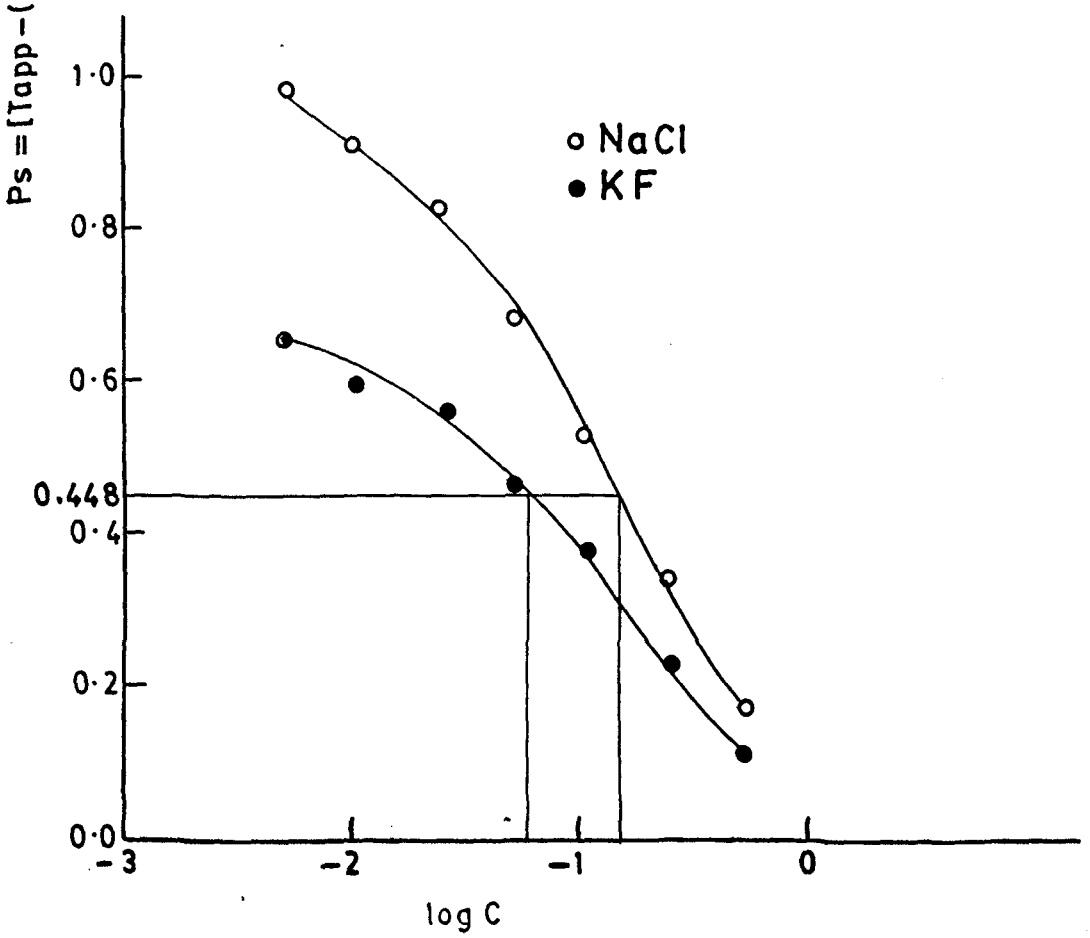


Fig 7 & 8: Plots of Ps vs log $C_1 + C_2/2$ for various electrolytes with pericardium

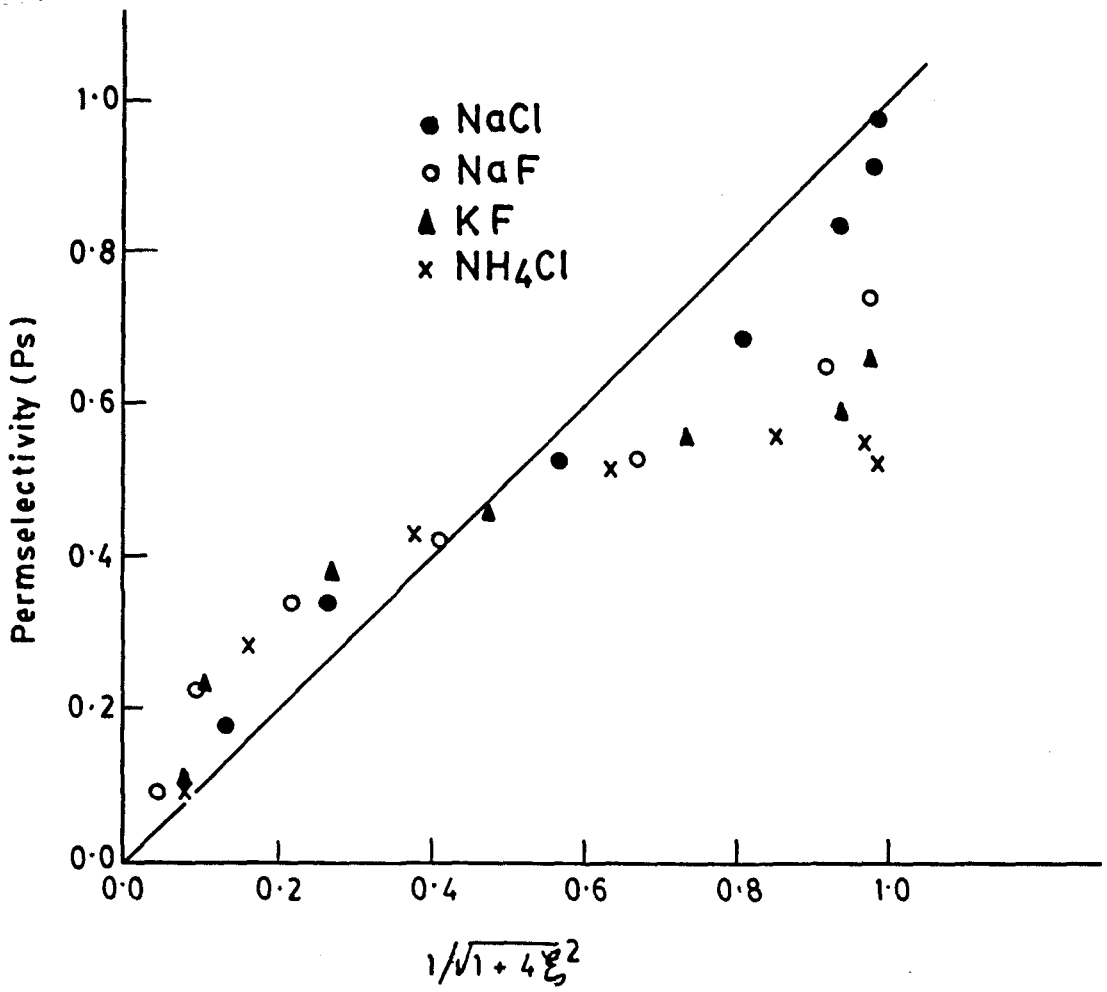


Fig.9: Plots of P_s vs $1/\sqrt{1+4\xi^2}$ for various electrolytes with pericardium

TABLE - 10

The values of Permselectivity (Ps) of membrane electrolyte systems for various electrolytes at different concentrations.

S.No.	Concentration C_2/C_1 (Moles/l)	Electrolytes NaCl	NaF	KCl	KF	NH4Cl
1	$1/1x10^{-1}$	0.178	0.094	0.0637	0.114	0.091
2	$5x10^{-1}/5x10^{-2}$	0.344	0.219	0.0312	0.229	0.279
3	$2x10^{-1}/2x10^{-2}$	0.533	0.343	0.118	0.377	0.431
4	$1x10^{-1}/1x10^{-2}$	0.689	0.423	0.029	0.462	0.509
5	$5x10^{-2}/5x10^{-3}$	0.837	0.527	0.0818	0.556	0.557
6	$2x10^{-2}/2x10^{-3}$	0.919	0.647	0.101	0.597	0.553
7	$1x10^{-2}/1x10^{-3}$	0.982	0.739	0.097	0.659	0.517

All Ps values are in negative.

TABLE - 11

Values of the effective fixed charge density of membrane electrolyte systems using different theories.

Membrane	Electrolyte	Kobatake et al. values			Kobatake and Kamo values	Nagasawa et al. values
		θ_c	θ_d	Ps vs log $\frac{C_1+C_2}{2}$	$\frac{1}{t_{app}}$ vs $1/C_1$	Em vs $1/C_1$
				øX	øX	øX
Peri-cardium	NaCl	10.7×10^{-2}	20.16×10^{-2}	15.14×10^{-2}	11.03×10^{-2}	4.11×10^{-2}
	NaF	4.93×10^{-2}	8.84×10^{-2}	5.01×10^{-2}	3.54×10^{-2}	1.29×10^{-2}
	KCl	3.94×10^{-2}	4.04×10^{-2}	———	1.89×10^{-2}	4.76×10^{-3}
	KF	6.58×10^{-2}	13.71×10^{-2}	6.07×10^{-2}	3.48×10^{-2}	1.64×10^{-2}
	NH_4Cl	6.71×10^{-2}	27.53×10^{-2}	8.91×10^{-2}	5.12×10^{-2}	1.90×10^{-2}

a curve between $\frac{1}{t_{app}}$ Vs $\frac{1}{C_1}$ which is shown in figure 10 and 11 and obtained the different value of charge density for pericardium with various electrolyte from the slope of line of the various curves given in figure 10 and 11.

$$\frac{1}{T_{app}} = \frac{1}{1-\alpha} + \frac{\gamma-1}{\gamma \ln \gamma} \quad \frac{\alpha}{1-\alpha} \left(\frac{\phi X}{C_1} \right) + o\left(\frac{1}{C_1} \right)^2 \ldots\ldots(14)$$

Nagasawa et al. (20) derived two limiting forms of expression for membrane potential (α) at the limit of low concentration, it was found that

$$-Em = \frac{RT}{F} \ln \frac{C_2}{C_1} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(15)$$

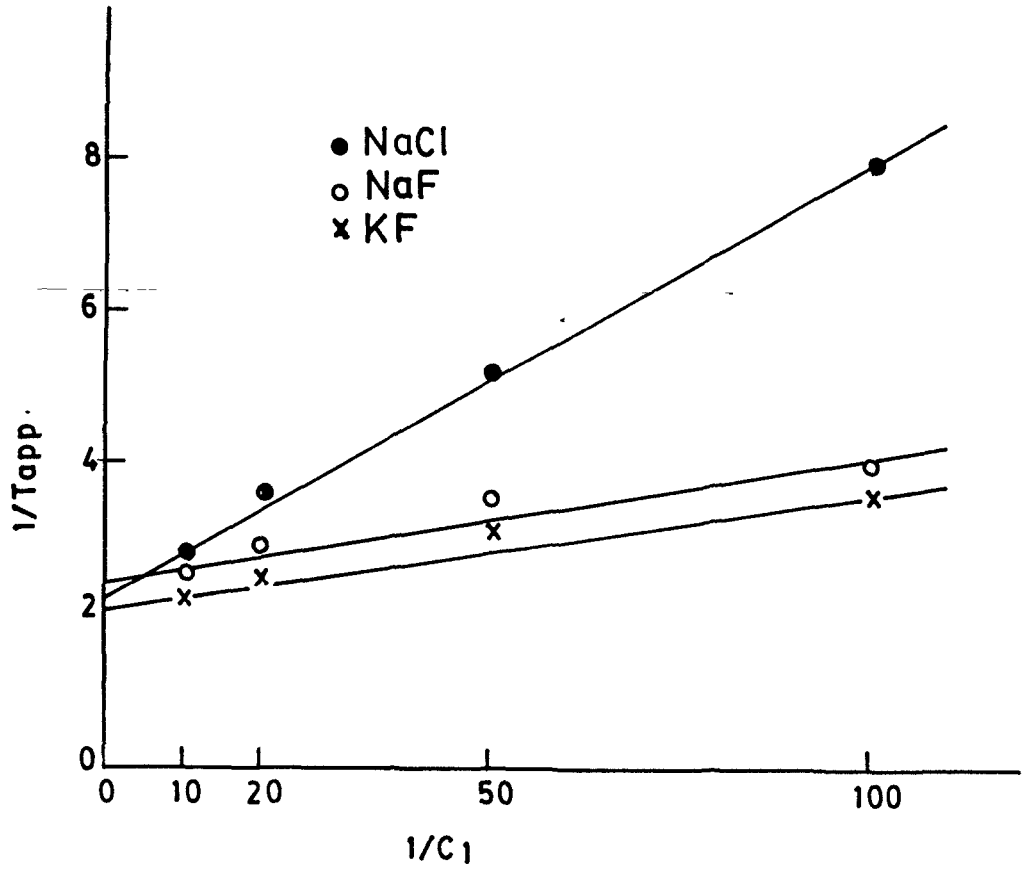
where Em is the membrane potential.

(b) At high concentrations of electrolyte, equation (16) is applicable as

$$-Em / \frac{\gamma-1}{\gamma} = \frac{RT}{F} \left(\frac{\phi X}{2} \right) \frac{1}{C_1} \qquad \ldots\ldots\ldots(16)$$

equation (16) predicts a linear relationship between $Em / \frac{\gamma-1}{\gamma}$ Vs. $\frac{1}{C_1}$ from which ϕX can also be determined.

The curves with various electrolytes for pericardium are shown in figure 12 and 13. The different values of ϕX derived from initial slope of various curves for pericardium with various electrolytes are shown in figure 12 and 13 . The value of ϕX derived in this way are given in table 11, with different electrolytes for the pericardial membrane.



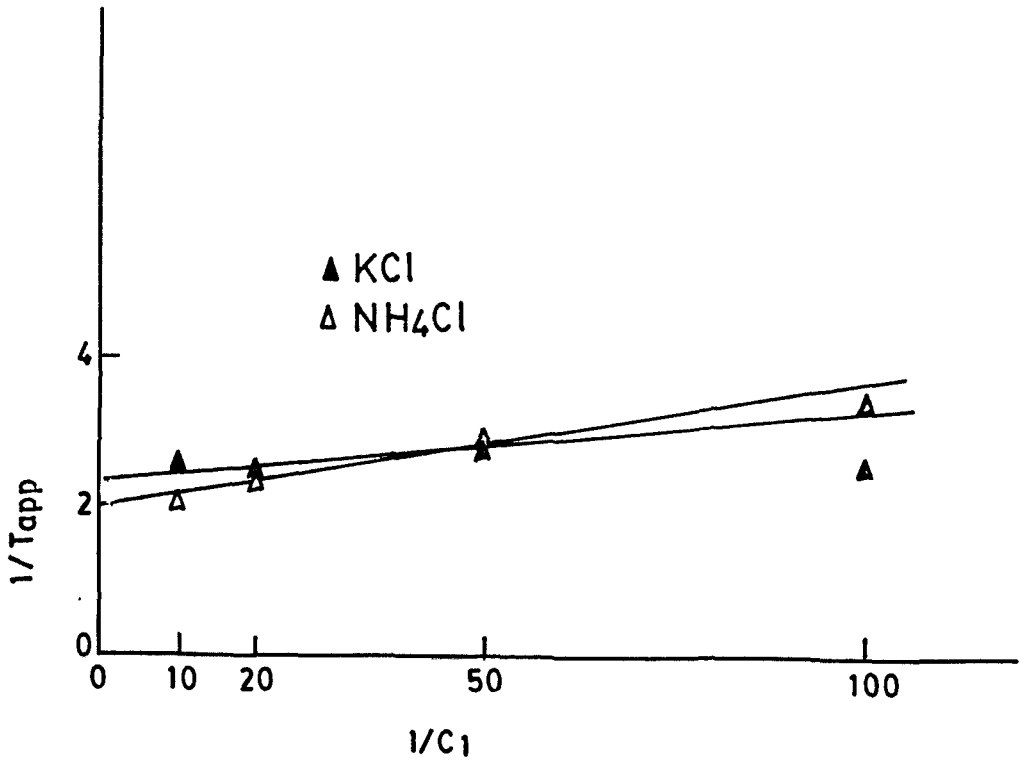
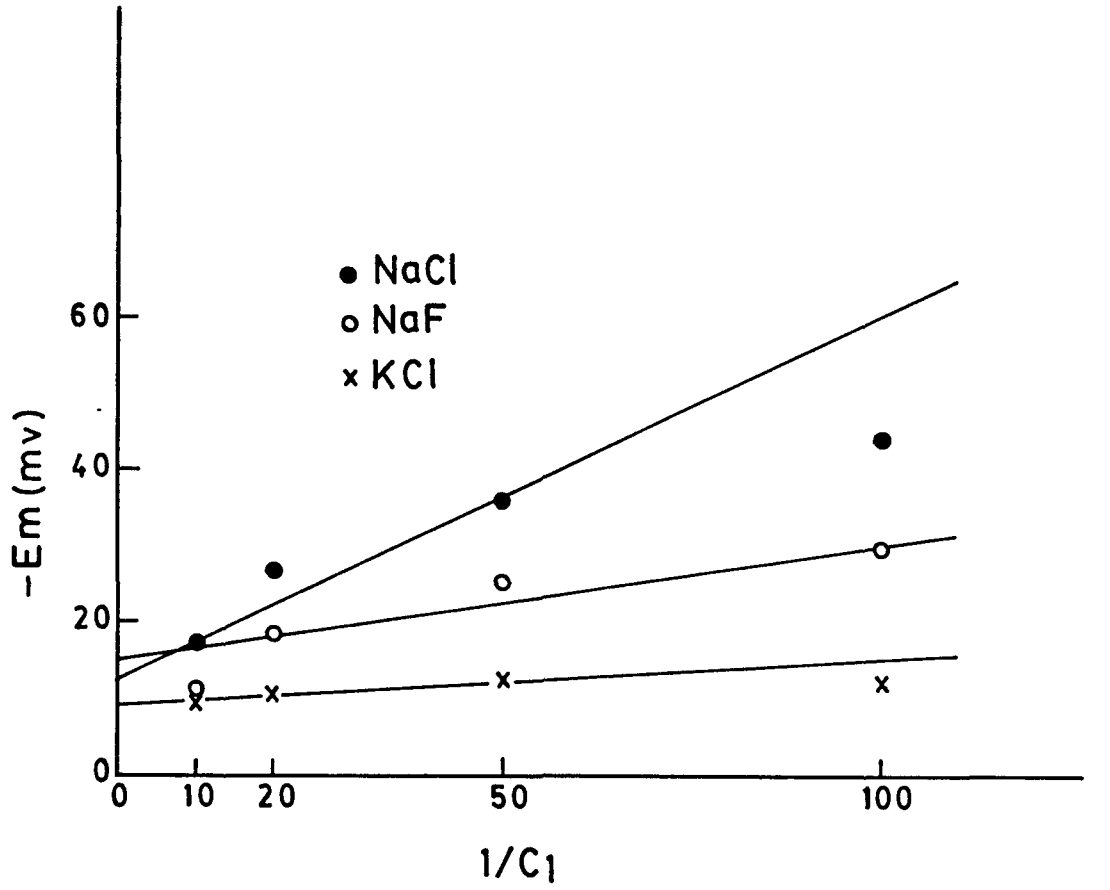


Fig.10&11 :Plots of 1/Tapp vs $1/C_1$ for various electrolytes with pericardium



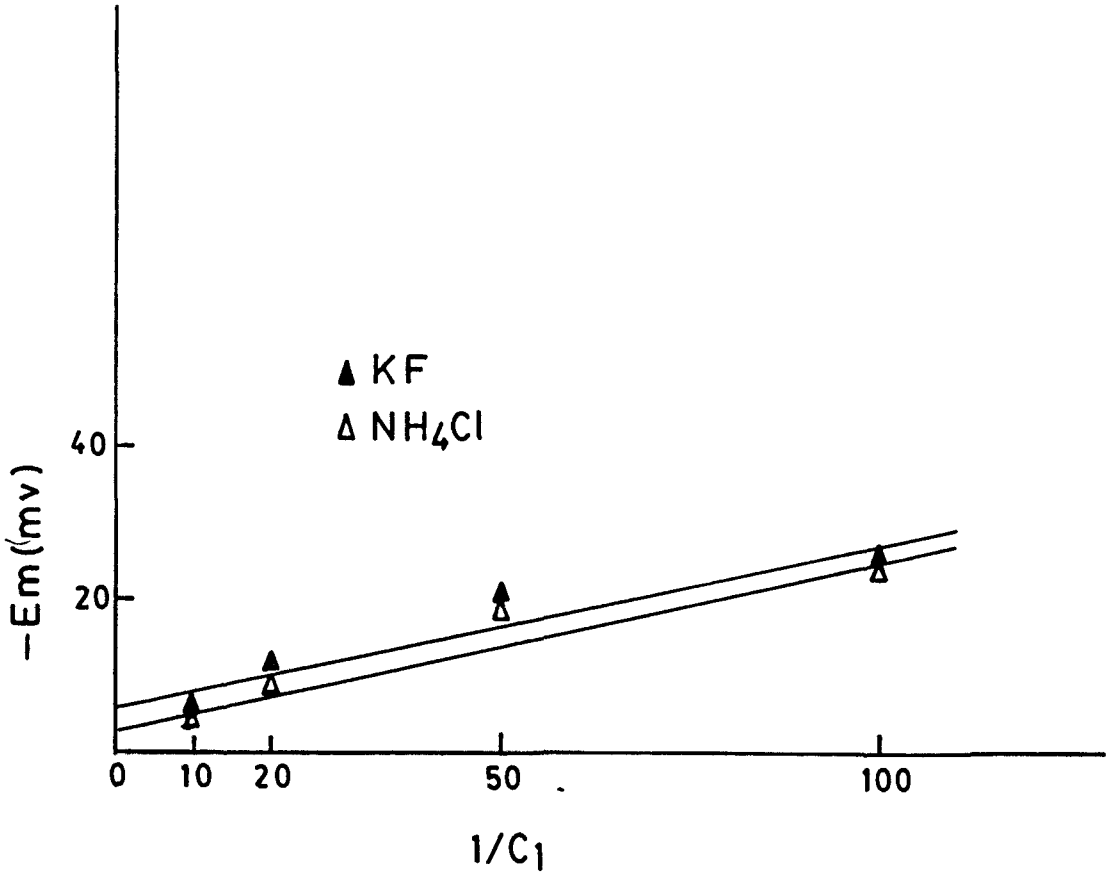


Fig.12&13:Plots of Em vs $1/C_1$ for various electrolytes with pericardium

The results of all these investigations show that the membrane potential can be determined with reasonable accuracy. When the pericardium is separating concentrated solutions the values of potential generated across pericardium are negative. This means that the membrane is anion selective and positively charged. Anion selectivity decreases as the concentration across the membrane is increased. The stepwise change in membrane potential or selectivity character of the membrane electrolyte system may be readily explained interms of structural changes produced in the electrical double layer at the interfaces.

Further, it is noted from the table 11 that the charge density values of the membrane electrolyte system are low. The values derived from different methods are almost same. A little difference may be attributed to the different procedure adopted. It is concluded from the results that the methods developed by Nagasawa et al. and Kobatake et al. are satisfactory for the evaluation of effective fixed charge density of the system.

It is also concluded from the results that t_{app} values for the different electrolytes increase by increasing the concentration of electrolytes across the pericardium. The results obtained are therefore, important in understanding the electrolyte transport across the Pericardium.

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